



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<p>(51) International Patent Classification 6 : C07K 14/68, 7/06, A61K 38/34</p>	<p>A1</p>	<p>(11) International Publication Number: WO 98/37097 (43) International Publication Date: 27 August 1998 (27.08.98)</p>
<p>(21) International Application Number: PCT/SE98/00270 (22) International Filing Date: 16 February 1998 (16.02.98) (30) Priority Data: 9700620-9 21 February 1997 (21.02.97) SE (71) Applicant (for all designated States except US): WAPHARM AB [SE/SE]; Trillvägen 13, S-905 90 Umeå (SE). (72) Inventors; and (75) Inventors/Applicants (for US only): WIKBERG, Jarl [SE/SE]; Stora Malmgatan 8, S-193 00 Sigtuna (SE). MUCENIECE, Ruta [LV/SE]; Sernanders väg 3/333, S-752 61 Uppsala (SE). MUTULIS, Feliks [LV/SE]; Tammi, Bandstolsvägen 20, S-756 48 Uppsala (SE). PRUSIS, Peteris [LV/SE]; Våktargatan 44E, S-754 22 Uppsala (SE). SCHIÖTH, Helgi-Birgir [IS/SE]; Lindsbergsgatan 3B, S-752 40 Uppsala (SE). (74) Agent: MIKSCHKE, Gerhard; Conimar AB, P.O. Box 2086, S-141 02 Huddinge (SE).</p>		<p>(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, GW, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE). Published With international search report.</p>
<p>(54) Title: MSH-RECEPTOR SUBTYPE SELECTIVE CYCLIC PEPTIDES</p> <div style="text-align: center;"> <p style="margin-left: 100px;">(I)</p> </div>		
<p>(57) Abstract</p> <p>Cyclic MC-receptor activating and/or blocking peptides of general formula (I) in which LRG is a large aminoacid and L is 26-29 membered linker connecting LRG and Trp are useful for treating conditions related to eating, body weight, motivation, learning, memory, behaviour, inflammation, body temperature, pain perception, blood pressure, heart rate, vascular tone, natriuresis, brain blood flow, nerve growth, placental development, aldosterone synthesis and release, thyroxine release, spermatogenesis, ovarian weight, prolactin and FSH secretion, uterine bleeding in women, sebum and pheromone secretion, blood glucose levels, intrauterine foetal growth, as well as other events related to parturition, and to afford neuroprotection. The novel peptides can also be labeled and be incorporated into pharmaceutical compositions.</p>		

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MSH-RECEPTOR SUBTYPE SELECTIVE CYCLIC PEPTIDES

FIELD OF THE INVENTION

- 5 The present invention relates to new MSH-receptor subtype selective cyclic peptide compounds having unique binding properties to the melanocortin (MC) receptors (MSH-receptors), and to their use in the treatment of conditions affected by those receptors in animals including man.

10 BACKGROUND OF THE INVENTION

- Melanocortic peptides (melanocortins) are natural peptide hormones in animals and man binding to and stimulating MC-receptors. Examples of melanocortins are α -MSH, β -MSH, γ -MSH, ACTH and their peptide fragments. α -MSH is
15 mainly known for its ability to regulate peripheral pigmentation (Eberle 1988), whereas ACTH is known to induce steroidogenesis (Simpson and Waterman, 1988). The melanocortic peptides also mediate a number of other physiological effects. They are reported to affect motivation, learning, memory, behaviour, inflammation, body temperature, pain perception, blood pressure,
20 heart rate, vascular tone, natriuresis, brain blood flow, nerve growth, placental development, aldosterone synthesis and release, thyroxine release, spermatogenesis, ovarian weight, prolactin and FSH secretion, uterine bleeding in women, sebum and pheromone secretion, blood glucose levels, intrauterine foetal growth, as well as other events related to parturition (Garrud
25 et al., 1974, Wiegant et al., 1979, O'Donahue et al., 1981, O'Donahue & Dorsa 1982, DeWied & Jolles 1982, Klein et al., 1985, Feng et al 1987, Lin et al 1987, Eberle, 1988, Gruber & Callahan 1989, DeWildt et al., 1995

- By use of molecular cloning genes encoding five different subtypes of
30 MC-receptors were identified (Chhajlani et al., 1993; Chhajlani and Wikberg, 1992; Gantz et al., 1993a & b; Mountjoy et al., 1992). The MC-receptors belong to the class of G-protein coupled receptors which are all built from a single peptide forming 7 transmembrane domains. The five MC-receptors are termed MC1, MC2, MC3, MC4 and MC5 and they all couple in a stimulatory fashion to

cAMP. Of these the MC2-receptor is the ACTH-receptor whereas the others constitute subtypes of MSH-receptors.

The MC1-receptor is present on melanocytes and melanoma cells (Low et al.,
5 1994, Siegrist & Eberle, 1995). Recent data also indicate that the MC1-receptor is expressed in limited areas (periaqueductal gray) of the rat and human brains (Xia et al. 1995), as well as in the testis (Vanetti et al. 1994). The MC2-receptor is the ACTH receptor and is present in the cortex of the adrenal gland. The MC3-receptor mRNA has been found in distinct areas of the brain, as well as in
10 placental and gut tissues (Gantz et al. 1993a, Desarnaud et al. 1994, Roselli-Rehfuss et al. 1993). The MC4-receptor has been found in the brain only (Gantz et al. 1993b; Mountjoy et al 1994). The MC5-receptor is expressed in the brain, as well as in several peripheral tissues (Chhajlani et al 1993; Gantz et al 1994; Griffon et al 1994; Labbú et al. 1994; Barrett et al. 1994; Fathi et al.
15 1995). More recent data indicate that all the 5 cloned MC-receptors have a wider tissue distribution (Chhajlani, 1996) than originally thought.

The five MC-receptors show unique affinities for the melanocortin peptides (Schiöth et al., 1995, Schiöth et al., 1996a,b,c). The MC1-receptor shows high
20 affinity for α -MSH, but lower affinities for β -MSH, γ -MSH, and ACTH. The MC2-receptor (the ACTH receptor) binds ACTH with high affinity, but does not bind the MSH peptides. The MC3-receptor shows slightly higher affinity for γ -MSH compared to β and α -MSH. The MC4-receptor shows slight preference for β -MSH over α -MSH, and a very low affinity for γ -MSH. The MC5- receptor
25 shows the same potency order for the MSH peptides as the MC1-receptor, although it binds the peptides with much lower affinities. The overall picture is that these peptides are all selective for the MC1-receptor (Schiöth et al., 1995, Schiöth et al., 1996a,b,c).

30 Natural melanocortin peptides show various effects not yet related to the different MC-receptor subtypes. It is believed that these effects are mediated by different MC-receptors and that many of them are mediated by the newly discovered MC3, MC4 and MC5 receptors. MC-receptor subtypes have, for instance, been attributed to the control of eating and body weight in agouti (Fan

et al. 1997). The wide distribution of MC4 receptors in the central nervous system also prompts them as a target for neuroprotective treatment.

MSH-receptors have been known as physiological entities since 1957. Binding sites for MSH/ACTH peptides have been identified in various brain and peripheral tissues (Hnatowich et al., 1989, Tatro & Reichlin, 1987, Lichtensteiger et al., 1993, Tatro & Entwistle, 1994). Peptide structure activity studies for these receptors have been performed on melanophores from lower vertebrates like *Rana pipiens* (frog), *Anolis carolinensis* (lizard) and *Xenopus laevis*. Receptor studies were later also performed by binding to melanoma cell lines. These test systems gave comparable results. It is now known that the data obtained with these systems refer to the MC1-receptor (Eberle, 1988). By using such test systems it was found that replacement of L-Phe with D-Phe in the MSH gave high potency and prolonged actions (Sawyer et al., 1980). Synthesised cyclic [Cys⁴, Cys¹⁰] α -MSH analogues were found to be potent melanotropes in skin pigmentation bioassays (Knittle et al., 1983, Sawyer et al., 1982). There exist 2 reports addressing potentially selective substances for the new receptor subtypes (MC3, MC4 and MC5). Hruby et al. synthesised cyclic lactam α -MSH (5-10) analogues (like SHU9119) which are putative MC4-receptor antagonists (Hruby et al., 1995). Another report suggested linear ACTH(4-10) to show some selectivity for MC3-, MC4- and MC5- receptors (Adan et al 1995).). However, more recent studies suggest these substances to be non-selective (Schiöth, HB et al. Peptides, 1997, 18, 1009-1013).

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OBJECTS OF THE INVENTION

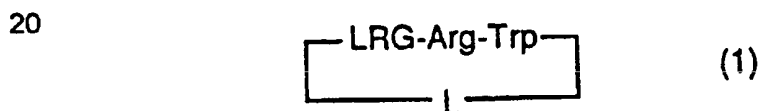
It is a principal object of the invention to devise means and methods to selectively activate or block the different MC-receptors, thereby eliciting pharmacological effects affecting conditions related to motivation, learning, memory, behaviour, inflammation, body temperature, pain perception, blood pressure, heart rate, vascular tone, natriuresis, brain blood flow, nerve growth, placental development, aldosterone synthesis and release, thyroxine release, spermatogenesis, ovarian weight, prolactin and FSH secretion, uterine bleeding in women, sebum and pheromone secretion, blood glucose levels,

intrauterine foetal growth, as well as other events related to parturition, and to afford neuroprotective effects, and for the treatment of eating disorders.

It is another object of the invention to provide chemical compounds selectively activating and/or blocking the various MC-receptors. It is a further object of the invention to provide means for administration of said compounds to animals or man. Still other objects of the invention will be evident from the following disclosure of the invention and the appended claims.

10 DISCLOSURE OF THE INVENTION

By careful engineering we have found the structural requirements for a cyclic peptide to afford selective high affinity binding to receptors of the MC3, MC4 and/or MC5 type. The structural requirements constitute an optimal ring size of the peptide cycle, and the inclusion of a large amino acid of proper structure, at the proper location in the peptide, as is described in the following. Thus, according to the present invention are disclosed cyclic MC-receptor activating and/or blocking peptides of the general formula (1):



in which LRG is large aminoacid and L is linker connecting LRG and Trp, thereby forming a cycle having from 26 to 29, preferably 29 members. By 26 to 29 members in this context is meant that the ring contains from 26 to 29 atoms.

'MC-receptor activating and/or blocking' denotes the capacity of a compound of the invention to activate and/or block certain MC receptor(s).

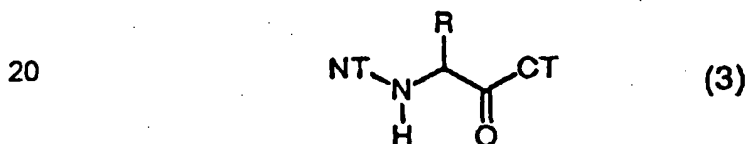
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A preferred aspect of the invention are cyclic MC-receptor activating and/or blocking peptides of the formula (1) wherein L is:



in which X is a non-cyclic peptide of two to three aminoacids, Y is a non-cyclic peptide of one to two aminoacids, A and B are non-cyclic peptides, each of from 0 to 5 aminoacids, and in which the cystein residues are connected by a disulphide bond. In formula (2) it is understood that neither of A, X, Y and B are
 5 connected to each other with covalent bond(s).

The term "aminoacid" as employed herein by itself or as part of another group refers to alanine, arginine, asparagine, aspartic acid, p-benzoyl-phenylalanine, β -cyclohexyl-alanine, cysteine, glutamic acid, glutamine, glycine, histidine, iso-
 10 leucine, leucine, lysine, methionine, β -(2-naphtyl)-alanine, norleucine, phenyl-alanine, proline, serine, threonine, tryptophan, tyrosine, valine, 3,4-dichlorophenylalanine, 4-fluorophenylalanine, 4-nitrophenylalanine, 2-thienylalanine, 3-benzothierylalanine, 4-cyanophenylalanine, 4-iodophenylalanine, 4-bromophenylalanine, 4,4'-biphenylalanine,
 15 pentafluorophenylalanine, β,β -diphenylalanine, in either D- or L-conformations, D-L-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid, as well as to compounds having the general structure (3):



in which R is H or CH_2R^1 , wherein R^1 is H, alkyl, substituted alkyl, heteroalkyl, substituted heteroalkyl, alkenyl, substituted alkenyl, heteroalkenyl, substituted
 25 heteroalkenyl, alkynyl, substituted alkynyl, heteroalkynyl, substituted heteroalkynyl, cycloalkyl, substituted cycloalkyl, cycloheteroalkyl, substituted cycloheteroalkyl, cycloalkenyl, substituted cycloalkenyl, cycloheteroalkenyl, substituted cycloheteroalkenyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, or functional group, NT is selected from H, functional group and
 30 bond to another aminoacid, CT is selected from functional group or bond to another aminoacid, the compounds according to formula (3) being in either D- or L-conformation.

The term "large aminoacid" as employed herein by itself or as part of another
 35 group refers to aminoacids in which R of formula (3) contains at least 14 atoms,

- preferably at least 15 atoms, with the D-form also being preferred and with phenylalanine being excluded. Most preferred are large aminoacids selected from the group of D- β -(2-naphtyl)-alanine, D-p-benzoyl-phenylalanine, D- β -cyclohexylalanine, D-3,4-dichlorophenylalanine, D-4-fluorophenylalanine, D-4-nitrophenylalanine, D-2-thienylalanine, D-3-benzothienylalanine, D-4-cyanophenylalanine, D-4-iodophenylalanine, D-4-bromophenylalanine, D-4,4'-biphenylalanine, D-pentafluorophenylalanine, D- β , β -diphenylalanine and D-L-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid.
- 5
- 10 The term "alkyl" as employed herein by itself or as part of another group includes a straight or branched hydrocarbon chain of up to 18, preferably from 1 to 8 carbon atoms, such as methyl, ethyl, propyl, isopropyl, tert-butyl, butyl, pentyl, hexyl, heptyl and octyl.
- 15 The term "heteroalkyl" as employed herein by itself or as part of another group refers to alkyl in which one or several carbon atoms are exchanged for heteroatom.
- The term "alkenyl" as employed herein by itself or as part of another group
- 20 includes a straight or branched hydrocarbon chain of up to 18 carbon atoms, preferably from 2 to 8 carbon atoms, comprising one or several carbon-carbon double bonds, such as propenyl, butenyl, pentenyl.
- The term "heteroalkenyl" as employed herein by itself or as part of another
- 25 group refers to alkenyl where one or several carbon atoms are exchanged for heteroatom.
- The term "alkynyl" as employed herein by itself or as part of another group refers to alkyl or alkenyl containing one or several carbon-carbon triple bonds.
- 30 The term "heteroalkynyl" as employed herein by itself or as part of another group refers to heteroalkyl or heteroalkenyl containing one or several carbon-carbon triple bonds.

The term "cycloalkyl" as employed herein by itself or as part of another group refers to cyclic hydrocarbons containing from 3 to 12 carbons, preferably 3 to 8 carbons, such as cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, cyclooctyl, which may optionally be fused with 1 or 2 cycles, each cycle being
5 independently selected from the group consisting of cycloalkyl, cycloheteroalkyl, cycloalkenyl, cycloheteroalkenyl, aryl and/or heteroaryl.

The term "cycloheteroalkyl" as employed herein by itself or as part of another group refers to cycloalkyl in which one or several carbon atoms are exchanged
10 for heteroatom.

The term "cycloalkenyl" as employed herein by itself or as part of another group refers to cycloalkyl containing one or several carbon-carbon double bonds, such as cyclopentenyl and cyclohexenyl.
15

The term "cycloheteroalkenyl" as employed herein by itself or as part of another group refers to cycloheteroalkyl where one or more bonds between carbons, carbon and heteroatom, or heteroatoms are double.

20 The term "aryl" as employed herein by itself or as part of another group refers to phenyl which may optionally be fused with 1 or 2 cycles which are independently selected from the group consisting of cycloalkyl, cycloheteroalkyl, cycloalkenyl, cycloheteroalkenyl, aryl, and/or heteroaryl.

25 The term "heteroaryl" as employed herein by itself or as part of another group refers to a 5- to 12-membered aromatic ring, preferably a 5- to 6-membered aromatic ring, which includes one or more heteroatoms, and which may optionally be fused with 1 or 2 cycles which are independently selected from the group consisting of cycloalkyl, cycloheteroalkyl, cycloalkenyl, cycloheteroalkenyl, aryl, and/or heteroaryl.
30

The term "substituted" refers to one or several hydrogens being substituted, independently, by alkyl, fluorinated alkyl, alkenyl, fluorinated alkenyl, alkynyl, fluorinated alkynyl, cycloalkyl, fluorinated cycloalkyl, cycloheteroalkyl, fluorinated cycloheteroalkyl, cycloalkenyl, fluorinated cycloalkenyl, cyclo-
35

heteroalkenyl, fluorinated cycloheteroalkenyl, aryl, fluorinated aryl, heteroaryl, fluorinated heteroaryl and/or functional group. Moreover, if a "substituted" structure is a cyclic structure fused with other cyclic structure(s) these latter cyclic structure(s) may also be substituted.

5

The term "halogen" as employed herein by itself or a part of another group refers to chlorine, bromine, fluorine and iodine with chlorine being preferred.

10 The term "heteroatom" as employed herein by itself or as part of another group refers to nitrogen, oxygen or sulphur, to which one or more hydrogens may be connected according to heteroatom valence; in the case of nitrogen one oxygen atom may be optionally connected to it by donor or acceptor bond, such as forming an N-oxide.

15 The term "functional group" as employed herein by itself or as part of another group refers to amino, alkylamino, dialkylamino, arylamino, arylazido, hetero-aryl amino, heteroarylazido, hydroxy, alkylhydroxy, fluorinated alkylhydroxy, cyano, carboxy, alkylcarboxy, arylcarboxy, guanidino, halogen, nitro, hydroxyl-amino, acyl, fluorinated acyl, nitroso, sulfonyl, sulfinyl, thio, alkylthio, or
20 arylthio.

The term "fused" as employed herein by itself or as part of another group refers to two or three cycles having one or more common atoms, the preferred maximum number of fused cycles being three.

25

Abbreviations used herein are listed below:

Gly = Glycine; Ala = Alanine; Val = Valine; Leu = Leucine; Ile = Isoleucine;
Ser = Serine; Cys = Cysteine; Thr = Threonine; Met = Methionine;
30 Phe = Phenylalanine; Tyr = Tyrosine; Trp = Tryptophan; Pro = Proline;
His = Histidine; Lys = Lysine; Arg = Arginine; Asp = Aspartic acid;
Glu = Glutamic acid; Asn = Asparagine; Gln = Glutamine; Nle = Norleucine;
Bpa = p-Benzoyl-phenylalanine; Nal = β -2-Naphtyl-alanine;
Cha = β -Cyclohexylalanine, 3,4-Dcp = 3,4-Dichlorophenylalanine; 4-Fpa =
35 4-Fluorophenylalanine; 4-Npa = 4-Nitrophenylalanine; Tha = 2-Thienylalanine;

Tic = D-L-1,2,3,4-Tetrahydroisoquinoline-3-carboxylic acid; 3-Bal = 3-Benzothienylalanine; 4-Ypa = 4-Cyanophenylalanine; 4-lph = 4-Iodophenylalanine; 4-Rpa = 4-Bromophenylalanine; Bip = 4,4'-Biphenylalanine; Pfp = Pentafluorophenylalanine; Dip = β , β -Diphenylalanine.

5

The letter "D" preceding the above abbreviations, e.g. as in "D-Nal" or "D-Phe", denotes the D-form of the amino acid. Absence of "D" indicates that the abbreviation is intended to refer to the L-form. In the following "Ac" refers to acetyl.

- 10 Particularly preferred according to the invention are the cyclic peptides HS005, HS006, HS007, HS010, HS011, HS012, HS014, HS964, as well as the other cyclic peptides listed below, the structural formulas of which are as follows:

HS005 Ac-Cys-Glu-His-D-Cha-Arg-Trp-Gly-Cys-NH₂

HS006 Ac-Cys-Glu-His-D-Bpa-Arg-Trp-Gly-Cys-NH₂

HS007 Ac-Cys-Arg-His-D-Nal-Arg-Trp-Gly-Cys-NH₂

HS010 Ac-Cys-Nle-Glu-His-D-Nal-Arg-Trp-Gly-Cys-NH₂

HS011 Ac-Cys-Glu-Ala-D-Nal-Arg-Trp-Gly-Cys-NH₂

HS012 Ac-Nle-Cys-Glu-His-D-Nal-Arg-Trp-Gly-Cys-NH₂

HS014 Ac-Cys-Glu-His-D-Nal-Arg-Trp-Gly-Cys-Pro-Pro-Lys-Asp-NH₂

HS009 Ac-Cys-Glu-His-D-Nal-Arg-Trp-Asp-Cys-NH₂



HS011 Ac-Cys-Glu-Ala-D-Nal-Arg-Trp-Gly-Cys-NH₂



HS015 Ac-Cys-Glu-Pro-D-Nal-Arg-Trp-Gly-Cys-NH₂



HS016 Ac-Cys-Arg-His-D-Nal-Arg-Trp-Gly-Cys-Asp-Arg-Phe-NH₂



HS017 Ac-Cys-Glu-Glu-D-Nal-Arg-Trp-Gly-Cys-NH₂



HS018 Ac-Cys-Glu-His-Gly-D-Nal-Arg-Trp-Cys-NH₂



HS019

Ac-Pro-Tyr-Arg-Cys-Glu-His-D-Nal-Arg-Trp-Gly-Cys-Pro-Pro-Lys-Asp-NH₂



HS020

Ac-Tyr-Val-Cys-Nle-Glu-His-D-Nal-Arg-Trp-Gly-Cys-Asp-Arg-Phe-NH₂



HS023 Ac-Cys-Gly-His-D-Nal-Arg-Trp-Gly-Cys-NH₂



HS024 Ac-Cys-Nle-Gly-His-D-Nal-Arg-Trp-Gly-Cys-NH₂



HS028

Ac-Cys-Glu-His-D-3,4-Dcp-Arg-Trp-Gly-Cys-Pro-Pro-Lys-Asp- NH₂


HS029

Ac-Cys-Glu-His-D-4-Fpa-Arg-Trp-Gly-Cys-Pro-Pro-Lys-Asp- NH₂


HS030

Ac-Cys-Glu-His-D-4-Npa-Arg-Trp-Gly-Cys-Pro-Pro-Lys-Asp- NH₂


HS031

Ac-Pro-Tyr-Cys-Nle-Arg-His-D-Nal-Arg-Trp-Gly-Cys-Pro-Pro-Lys-Asp- NH₂


HS032

Ac-Cys-Nle-Arg-His-D-Nal-Arg-Trp-Gly-Cys-Pro-Pro-Lys-Asp- NH₂
HS040 Ac-Cys-Nle-Glu-His-D-Nal-Arg-Trp-Cys-Lys-Pro-Val- NH₂
HS050 Ac-Ser-Tyr-Cys-Nle-Arg-His-D-Nal-Arg-Trp-Gly-Cys- NH₂
HS051 Ac-Ser-Tyr-Cys-Nle-Glu-His-D-Nal-Arg-Trp-Gly-Cys- NH₂
HS052 Ac-Cys-Nle-Arg-Glu-D-Nal-Arg-Trp-Gly-Cys- NH₂
HS053 Ac-Cys-Nle-Lys-His-D-Nal-Arg-Trp-Gly-Cys- NH₂
HS054 Ac-Cys-Phe-Arg-His-D-Nal-Arg-Trp-Gly-Cys- NH₂


HS055 Ac-Cys-Asn-Arg-His-D-Nal-Arg-Trp-Gly-Cys- NH₂



HS058 Ac-Cys-Nle-Arg-His-D-Nal-Arg-Trp-Gly-Cys-Tyr- NH₂



HS059 Ac-Cys-Nle-Arg-His-D-Nal-Arg-Trp-Tyr-Cys- NH₂



HS060 Ac-Cys-Nle-Arg-His-D-3,4-Dcp-Arg-Trp-Gly-Cys- NH₂



HS061 Ac-Cys-Nle-Arg-His-D-4-Fpa-Arg-Trp-Gly-Cys- NH₂



HS062 Ac-Cys-Nle-Arg-His-D-4-Npa-Arg-Trp-Gly-Cys- NH₂



HS063 Ac-Cys-Nle-Arg-His-D-Cha-Arg-Trp-Gly-Cys- NH₂



HS064 Ac-Cys-Nle-Arg-His-D-Bpa-Arg-Trp-Gly-Cys- NH₂



HS069 Ac-Cys-Nle-Arg-His-D-Tic-Arg-Trp-Gly-Cys- NH₂



HS071

Ac-Cys-Glu-His-D-Tic-Arg-Trp-Gly-Cys-Pro-Pro-Lys-Asp- NH₂



HS072

Ac-Cys-Nle-Arg-His-D-Nal-Arg-Trp-Gly-Cys-Pro-Pro-Lys-Tyr- NH₂



HS073

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HS074

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HS078

Ac-Cys-Glu-His-D-4-lph-Arg-Trp-Gly-Cys-Pro-Pro-Lys-Asp- NH₂


HS082

Ac-Cys-Glu-His-D-4-Rpa-Arg-Trp-Gly-Cys-Pro-Pro-Lys-Asp- NH₂





HS083

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HS085

Ac-Cys-Glu-His-D-Dip-Arg-Trp-Gly-Cys-Pro-Pro-Lys-Asp- NH₂
HS086 Ac-Cys-Nle-Glu-His-D-3-Bal-Arg-Trp-Gly-Cys- NH₂
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HS091 Ac-Cys-Nle-Glu-His-D-4-lph-Arg-Trp-Gly-Cys- NH₂


HS095 Ac-Cys-Nle-Glu-His-D-4-Rpa-Arg-Trp-Gly-Cys- NH₂

HS096 Ac-Cys-Nle-Glu-His-D-4-Bip-Arg-Trp-Gly-Cys- NH₂

HS097 Ac-Cys-Nle-Glu-His-D-Pfp-Arg-Trp-Gly-Cys- NH₂

HS098 Ac-Cys-Nle-Glu-His-D-Dip-Arg-Trp-Gly-Cys- NH₂

HS964 Ac-Cys-Glu-His-D-Nal-Arg-Trp-Gly-Cys- NH₂

Also in this patent is mentioned substance HS963 which is not part of the invention. HS963 is included for the sole purpose of comparison, in order to demonstrate the unique properties of the compounds of the invention

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HS963 Ac-Cys-Glu-His-D-Phe-Arg-Trp-Gly-Cys-NH₂

25 Methods for synthesis of above compounds are disclosed in Examples 1-10 and 11-56.

30 The compounds of the present invention bind preferentially to particular MC-receptor subtypes. This binding capacity becomes evident when tested on recombinant human MC-receptors by use of methods described in the literature (see Schiöth et al., Eur. J. Pharmacol., Mol. Pharm. Sect. 1995, 288, 311 and Pharmacol. & Toxicol. 1996, 79, 161). In these tests the ability of the compounds to compete for the binding of [¹²⁵I]-labelled NDP-MSH ([Nle⁴, D-Phe⁷]α-MSH) to recombinant human MC-receptor subtypes expressed in COS-1 cells were evaluated, as described in Example 10. By these assays the

dissociation constants (K_i s) were determined for a number of the compounds according to the invention (Table 1).

Table 1. Binding capacities of cyclic MC-receptor activating and/or blocking peptides according to the invention

	Compound	MC1 K_i (nM)	MC3 K_i (nM)	MC4 K_i (nM)	MC5 K_i (nM)
10	NDP-MSH	0.11	0.47	2.9	5.5
	α -MSH	0.033	21	640	8200
	β -MSH	1.2	13	380	14000
	γ 1-MSH	2.7	7.1	29000	43000
	ACTH(1-39)	2.5	87	690	17000
15	HS005	8200	5000	1000	8600
	HS006	610	120	38	80
	HS007	230	17	7.0	53
	HS010	280	82	225	344
20	HS011	74	21	5.0	7.1
	HS012	210	130	27	130
	HS014	23	13	0.95	140
	HS963	470	3100	1800	6000
	HS964	1500	280	23	160
25	HS015	170	280	240	180
	HS016	28	5.7	4.4	46
	HS017	31000	32000	3600	15000
	HS019	110	47	5.8	1100
	HS020	37	61	15	90
30	HS024	19	4.6	0.29	3.3
	HS028	59	74	0.95	210
	HS029	120	960	22	2600
	HS030	770	410	20	730
	HS031	6.5	15	4.7	8.5
35	HS032	3.4	2.1	0.20	2.8

	HS040	100	110	22	47
	HS053	42	9.1	3.8	420
	HS055	1.3	5.3	0.75	7.1
	HS058	27	13	2.7	5.6
5	HS059	94	88	13	11

In Table 1 the K_i -values for non-labeled NDP-MSH and the natural MSH-peptides are given for comparison, the affinity profile for these peptides being MC1>MC3>MC4>MC5, which is a pattern close to that found for most previously known MC-receptor active compounds (Schiöth et al. 1995, 1996b). As is evident from Table 1 the compounds of the invention show an affinity profile substantially different from that of known MC-receptor activating and/or blocking peptides, with selectivities balanced between MC1, MC3, MC4 and MC5-receptors, the affinity profile for individual compounds according to the invention being balanced towards one, two or a few of the MC-receptor subtypes. Besides having unique affinity profiles the compounds also show remarkably high affinities for specific subtypes of the MC-receptors. These properties of the compounds of the invention are particularly desirable, in particular in conjunction with the administration of the compounds of the invention to animals and/or man.

In this context it should be stressed that the present invention comprises the combination of the optimal ring size of the cyclic peptide, being 26 to 29 membered, with the inclusion of large aminoacid (LRG) according to formula (1), which in general give a compound with unique and novel properties. The unique and novel properties consist, on one hand, of a preferential (selective) ability of a compound of the invention to bind to one or several of the MC3, MC4 and MC5 receptors compared to its ability to bind to the MC1 receptor. The novel property consist, on the other hand, in addition on a very high affinity of many of the compounds of the invention for one or several of the MC3, MC4 and MC5 receptors, concomitantly with its preferential (selective) binding ability mentioned in the aforementioned sentence.

In the present patent the MC3-selectivity of a compound is defined as the ratio of the K_i of the compound for an MC1 receptor (K_i -MC1) over the K_i of the

compound for the MC3 receptor (K_i -MC3), the K_i values being measured as described in Example 10 using the method described by Schiöth et al. 1995 and 1996b, hence:

$$\text{MC3-selectivity} = \frac{K_i\text{-MC1}}{K_i\text{-MC3}}$$

In the present patent is also defined the MC4-selectivity of a compound as the ratio of the K_i of the compound for an MC1 receptor (K_i -MC1) over the K_i of the compound for the MC4 receptor (K_i -MC4), the K_i values being measured as described in Example 10 using the method described by Schiöth et al. 1995 and 1996b, hence:

$$\text{MC4-selectivity} = \frac{K_i\text{-MC1}}{K_i\text{-MC4}}$$

Moreover, in the present patent is also defined the MC5-selectivity of a compound as the ratio of the K_i of the compound for an MC1 receptor (K_i -MC1) over the K_i of the compound for the MC5 receptor (K_i -MC5), the K_i values being measured as described in Example 10 using the method described by Schiöth et al. 1995 and 1996b, hence:

$$\text{MC5-selectivity} = \frac{K_i\text{-MC1}}{K_i\text{-MC5}}$$

A compound is herein defined as being selective for the MC3 receptor when the above mentioned ratio "MC3-selectivity" is preferably at least 3, somewhat more preferably at least 5, more preferably at least 10, even more preferably at least 20 and most preferably at least 30.

A compound is herein defined as being selective for the MC4 receptor when the above mentioned ratio "MC4-selectivity" is preferably at least 3, somewhat

more preferably at least 5, more preferably at least 10, even more preferably at least 20 and most preferably at least 30.

5 A compound is herein defined as being selective for the MC5 receptor when the above mentioned ratio "MC5-selectivity" is preferably at least 3, somewhat more preferably at least 5, more preferably at least 10, even more preferably at least 20 and most preferably at least 30.

10 By a "very high affinity" of a compound of the invention for an MC3 or MC4 or MC5 receptor is in the present patent meant a K_i value for the respective receptor being preferably less than 300 nM, somewhat more preferably being less than 100 nM, more preferably being less than 30 nM, even more preferably being less than 10 nM and most preferably being less than 3 nM; the K_i value being measured as described in Example 10 using the method
15 described by Schiöth et al. 1995 and 1996b.

The MC3-selectivity and/or MC4-selectivity and/or MC5-selectivity, including the optional very high affinity of a compound of the invention for the MC3 and/or MC4 and/or MC5 receptor is a very desired property as this avoids side
20 effects of the compound of the invention by not causing actions on the MC1 receptor (e.g. effects on skin pigmentation).

Prior to the disclosure of the present invention no compound existed that showed MC3-selectivity and/or MC4-selectivity and/or MC5-selectivity, and that
25 optionally also showed very high affinity for the MC3 and/or MC4 and or MC5 receptor.

The compounds of the invention can be used for the treatment and diagnosis of diseases, disorders and/or pathological conditions in an animal, in particular in
30 a mammal, but they are most preferably used for these purposes in man.

In such treatment or diagnosis the compound of the invention is administered in form of a pharmaceutical composition comprising a pharmaceutical acceptable carrier and, optionally, tableting agents, wetting agents, binders
35 and fillers, preservatives, such as antioxidants and anti-microbial agents,

5 buffers and salts. Preferred carriers comprise injection media, particularly water and other conventional media for injection. The compositions are administered by any conventional route including the oral, enteral, rectal and parenteral routes. Parenteral routes comprise intravenous, intramuscular, subcutaneous and peritoneal injection. The compounds of the invention may also be administered by inhalation, as nasal spray, and topically on the skin. They may also be administered epidurally, intrathecally and intracerebro-ventricularly.

10 In particular the pharmaceutical composition containing a pharmacologically effective amount of a compound of the invention is administered to an animal, in particular man, for diagnosis, prevention or therapeutic treatment of diseases, in particular conditions involving MC3- and/or MC4- and/or MC5-receptors. Examples of such MC3- and/or MC4- and/or MC5-receptor related conditions that are positively affected by administration of the
15 compounds of the invention are fever, pain, chronic inflammatory diseases, memory disturbances in particular in elderly people, including Alzheimer's disease. Moreover positive effects are obtained on the regeneration of nerves after nerve injuries, on psychomotor functions, in particular positive effects on pathological psychomotor functions of psychiatric conditions such as e.g.
20 catatonic conditions. The compounds of the invention are also be used for mediating anti-epileptic, anti-inflammatory and anti-pyretic effects, and for modulating signaling functions in both the brain and the periphery. Another important use of the compounds of the invention constitutes the treatment of weight disorders (e.g. overweight and underweight), in particular when the
25 weight disorder is related to an eating disorder, such as excessive food intake, reduced food intake, bulimia and/or anorexia, with respect to the latter in particular anorexia nervosa, of humans. Particularly useful for treatment of such disorders are compounds HS007, HS011, MS012, HS014, HS024, HS028 and HS964 due to the fact that they are balanced in their selectivities towards the
30 MC4-receptor.

A particularly important aspect of the invention is the use of the compounds of the invention for treatment of eating disorders related to underweight, cachexia or anorexia of any cause in humans. In these conditions the administration of a
35 compound of the invention will increase food intake, which improves the

patients general condition, increases or restores their body weight and prolong their life. In particular the administration of the compound of the invention is beneficial in elderly patients, in cancer patients, and in patients treated with cancer chemotherapeutics, as these patients often suffers from lack of appetite, that often lead to decreased food intake and severe underweight. Yet another important embodiment of the invention is the administration of the compound of the invention to an animal to increase its rate of growth. In particular the latter is desired in animal breeding for meat production. Very particular embodiments of the present invention constituting the administration of HS014 and HS028 for increasing food intake, for increasing body weight and for increasing rate of growth are given in Examples 57 and 58.

Other important uses of the compounds of the invention are for treatment of disturbances in: 1) placental development, 2) aldosterone synthesis and release, 3) thyroxin release, 4) spermatogenesis, 5) prolactin and FSH secretion, 6) sebum and/or pheromone secretion, 7) blood glucose levels, 8) natriuresis, and 9) intrauterine foetal growth. Moreover, compounds of the invention may be used for the treatment of uterine bleeding in women. Other important uses constitute control of blood pressure, heart rate, vascular tone and brain blood flow, and to afford neuroprotection.

Pharmacologically effective amounts may vary from 0.001 mg/day/kg body weight to 1,000 mg/day/kg body weight; however, lower amounts may be effective, in particular if delivered locally. The compounds of the invention have low toxicity and are well tolerated.

For analytical and diagnostic purposes the compounds of the present invention can be used in radioactive form, including radioactive labels. In particular the compounds of the invention may be manufactured so as to incorporate radioactive iodine or tritium, or any other suitable radio nuclide. Such a radioactively labeled compound can be used in radioligand binding for the quantification of specific melanocortin receptors, for the analysis of dissociation constant (K_d s or $K_{d,s}$) of drugs competing with specific subtypes of melanocortin receptors, and for the localization of MC-receptors in tissues and tissue sections e.g. by use of receptor autoradiographic techniques. Principles of

radioligand binding and receptor autoradiography are well known in the art. As an alternative the compound may be labeled with any other type of label that allows detection of the substance, e.g. a fluorescent label or biotin, and the resulting compound be used for the similar purpose as the radioactively labeled compound.

The compounds of the invention can also be manufactured so as to incorporate a group that can be activated by light, in particular UV-light, the purpose with such activation being to obtain a compound useful for covalent labeling of MC-receptor by use of the photoaffinity labeling technique. Photoaffinity labeling is a technique well known in the art which in the present context is useful for elucidating the structure and topological organisation of the MC-receptors. Thus photoactive derivatives of the compounds of the invention are also part of the present invention. Moreover, preferably photoactive derivatives of the compounds of the invention may also be made to incorporate an easily detectable group or label, such as e.g. a radioactive atom, a fluorescent group and/or biotin. (For further details in regard of photoaffinity labeling see Leeb-Lundberg et al J. Biol. Chem. 1984, 259, 2579 and Scimonelli & Eberle FEBS Lett. 1987, 226, 134.)

The compounds of the invention can be labeled with gamma and/or positron emitting isotope(s). Such labeled compounds constitute very specific embodiments of the invention and may be administered systematically, or locally, to an animal, preferably a human. These labeled compounds are useful for imaging the in vivo levels and/or localization of MC-receptors by the use of well known techniques among which may be mentioned Scintigraphy, Positron Emission Tomography (PET) and Single Photon Emission Computed Tomography (SPECT). Using such methods information on the distribution and/or quantities of the specific MC-receptors in tissues of the animal or human subject to the investigation is obtained, and such information is of value for diagnosis of disease, in particular functional disturbances in the brain related to MC-receptors.

Agonist and antagonist activities of the compounds of the invention may be evaluated by various methods known in the art. Examples of such methods are

measurement of second messenger responses, in particular cAMP, the use of modified cell systems yielding colour reaction upon accumulation of second messenger elements such as cAMP, e.g. as described by Chen et al. 1995 (Anal Biochem. 1995, 226, 349-54), Cytosensor Microphysiometer techniques (see Boyfield et al. 1996), or the study of physiological effects caused by the compounds of the invention may be applied by using the compounds of the invention alone, or in combination with natural or synthetic MSH-peptides.

The compound of the invention may be delivered to the preferred site in the body, such as e.g. to the brain, by using a suitable drug delivery system. Drug delivery systems are well known in the art. For example the compound of the invention may be coupled to a carrier molecule making it lipophilic (see e.g. Toth, I., J. Drug. Targeting, 1994, 2, 217-239; Patel et al., Bioconjugate Chem., 1997, 8, 434-441). Other technologies that can be used to deliver the compound of the invention to the desired site in the body are vector mediated carrier systems (see e.g. Pardridge, WM, Pharmacol. Toxicol. 1992, 71, 3-10; Saito, Y et al. Proc. Natl. Acad. Sci. USA 1995, 92, 10227-10231; Wu, D and Pardridge, WM J. Pharmacol. Exp. Ther. 1996, 279, 77-83). Yet other example of drug delivery technologies useful for the compounds of the present invention is the conjugation of the compound of the invention to an active molecule capable of being transported through a biological barrier (see e.g. Zlokovic, BV., Pharmaceutical Research 1995, 12, 1395-1406). A specific example constitutes the coupling of the compound of the invention to fragments of insulin to achieve transport across the blood brain barrier (Fukuta, M et al. Pharmaceutical Res. 1994, 11, 1681-1688). For general reviews of technologies for drug delivery suitable for the compounds of the invention see Zlokovic, BV, Pharmaceutical Res. 1995, 12, 1395-1406 and Pardridge, WM, Pharmacol. Toxicol. 1992, 71, 3-10.

The present invention also relates to a pro-drug which after the administration to an animal, including a human, is converted to a compound of the invention. A pro-drug of the compound of the invention can be used for the same purposes as described above for the compounds of the invention, as well as is disclosed in the Examples given below.

The compound of the present invention can be covalently or non-covalently bound to one or several of other optional molecule(s) of any desired structure(s); the thus formed modified compound or complex can be used for the same purposes as described above for the compounds of the invention, as well as is disclosed in the Examples given below.

In the following the invention will be described in greater detail by reference to a number of preferred embodiments which however are only given for purposes of illustration and must not be considered to limit the invention in any way.

10

EXAMPLES

Abbreviations. DMF = N,N-Dimethylformamide; DMSO = Dimethylsulfoxide; Fmoc-Cys(Trt)-OPfp = 9-Fluorenylmethoxycarbonyl-S-trityl-L-cysteine pentafluorophenyl ester; HOAt = 1-Hydroxy-7-azabenzotriazole; HOBt = 1-Hydroxybenzotriazole; Fmoc-Gly-OPfp = 9-Fluorenylmethoxycarbonyl-glycine pentafluorophenyl ester; Fmoc-Trp(Boc)-OH = 9-Fluorenylmethoxycarbonyl-(N-tert-butyloxycarbonyl)-L-tryptophan; HATU = O-(7-Azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate; Fmoc-Arg(Pbf)-OH = 9-Fluorenylmethoxycarbonyl-(N⁹-2,2,4,6,7-pentamethyldihydro benzofuran-5-sulfonyl)-L-arginine; Fmoc-D-Cha-OH = 9-Fluorenylmethoxycarbonyl-β-cyclohexyl-D-alanine; Fmoc-His(Trt)-OH = 9-Fluorenylmethoxycarbonyl-N^{im}-trityl-L-histidine; Fmoc-Glu(OBu^t)-OPfp = 9-Fluorenylmethoxycarbonyl-(γ-tert-butyl ester)-L-glutamic acid pentafluorophenyl ester. MeCN = Acetonitrile. Fmoc-D-Nal-OH = 9-Fluorenylmethoxycarbonyl-β-2-naphtyl-D-alanine. Fmoc-Nle-OPfp = 9-Fluorenylmethoxycarbonyl-L-norleucine pentafluorophenyl ester.

Example 1. *Synthesis of cyclo(S-S)-[Ac-Cys⁴, D-Cha⁷, Cys-NH²¹¹] α-MSH(4-10) trifluoroacetate (HS005).* The peptide sequence was assembled on a solid support using the Pioneer peptide synthesis system from PerSeptive Biosystems.

Start procedure: 250 mg (0.05 mmole) of [5-(4-Fmoc-aminomethyl-3,5-dimethoxy)valeric acid attached to polyethylene-graft polystyrene support

(Fmoc-PAL-PEG-PS, capacity 0.2 mmole/g) was placed in a peptide synthesis column. The Fmoc group was then removed by 5 min treatment with 20 % piperidine in DMF, followed by a wash of the support with DMF.

- 5 *Amino acid coupling.* After completion of the start procedure the resin was subjected to repeated aminoacid coupling cycles, each cycle consisting of 30 - 60 min circulation of appropriate reagents (as detailed below) dissolved in 4 ml DMF through the column, followed by washing with DMF, 5 min treatment with 20 % piperidine in DMF, and then again washing with DMF before the start of
- 10 the next cycle. For synthesis of HS005 eight cycles were applied using reagents and treatment times as follows (in order): 1) Fmoc-Cys(Trt)-OPfp (150.4 mg, 0.2 mmole) and HOAt (27.2 mg, 0.2 mmole) (30 min), 2) Fmoc-Gly-OPfp (92.7 mg, 0.2 mmole) and HOAt (27.2 mg, 0.2 mmole) (30 min), 3) Fmoc-Trp(Boc)-OH (105.3 mg, 0.2 mmole), HATU (76.0 mg, 0.2 mmole) and N,N-diisopropylethylamine (0.17 ml, 1.0 mmole) (30 min), 4) Fmoc-Arg(Pbf)-OH
- 15 (129.8 mg, 0.2 mmole), HATU (76.0 mg, 0.2 mmole) and N,N-diisopropylethylamine (0.17 ml, 1.0 mmole) (60 min), 5) Fmoc-D-Cha-OH (78.7 mg, 0.2 mmole), HATU (76.0 mg, 0.2 mmole) and N,N-diisopropylethylamine (0.17 ml, 1.0 mmole) (60 min), 6) Fmoc-His(Trt)-OH (123.9 mg, 0.2 mmole),
- 20 HATU (76.0 mg, 0.2 mmole) and N,N-diisopropylethylamine (0.17 ml, 1.0 mmole) (60 min), 7) Fmoc-Glu(OBut)-OPfp (118.3 mg, 0.2 mmole) and HOAt (27.2 mg, 0.2 mmole) (30 min), 8) Fmoc-Cys(Trt)-OPfp (150.4 mg, 0.2 mmole) and HOAt (27.2 mg, 0.2 mmole) (60 min).
- 25 *Release and deprotection.* After the last cycle in a final procedure acetic anhydride (0.094 ml, 1.0 mmole) and N,N-diisopropylethylamine (0.17 ml, 1.0 mmole) dissolved in 4 ml DMF was circulated through the column for 10 min. The support was then washed with DMF, followed by a methanol wash, and dried in vacuo. The dried resin was treated with 5 ml of deprotection mixture
- 30 (trifluoroacetic acid - phenol - anisole - 1,2-ethanedithiol - water, 82:2:2:2) for 2 hours at room temperature. It was filtered, washed on the filter with trifluoroacetic acid, the filtrates united and concentrated in vacuo at room temperature. Dry ether was added and the precipitate formed was filtered off and washed on the filter with ether, then dried in vacuo over KOH. Yield 41.8
- 35 mg. HPLC data (2 x 250 mm column, Vydac RP C18, 90A, 201HS1010): k'

(main substance) = 4.26 (a gradient in 30 minutes from 18 % MeCN to 60 % MeCN in 0.1 % trifluoroacetic acid).

Formation of disulphide. The raw peptide was dissolved in 3 ml DMSO and
5 placed under argon into a thermostat at 65°C for 36 h. The solvent was then
evaporated at room temperature in vacuo. The residue was dissolved in 1 ml of
60 % MeCN in water and the solution divided into three portions and placed
into centrifuge tubes, each of them then being diluted with 0.1 % aqueous
trifluoroacetic acid to 1.5 ml volume. The clear solutions obtained upon
10 centrifugation were used for semi-preparative HPLC (10 x 250 mm column,
Vydac RP C18, 90A, 201HS1010, eluate - 24 % acetonitrile in water + 0.1 %
trifluoroacetic acid, detection at 240 nm. Fractions containing the main peak
were pooled and lyophilized. A white powder was obtained in a yield of 8.2 mg
(14 %). R_f 0.49 (1-butanol - pyridine - acetic acid - water, 4:1:1:2). Mass
15 spectrometry: m/e = 1082.3.

Example 2. *Synthesis of cyclo(S-S)-[Ac-Cys⁴, D-Bpa⁷, Cys-NH₂¹¹] α -MSH(4-11) trifluoroacetate. (HS006).* The same approach as in Example 1 was used,
the starting procedure being exactly identical.

20 *Amino acid coupling.* Eight amino acid attachment cycles were then applied
using the approach of Example 1; the reagents and treatment times being (in
order) 1) Fmoc-Cys(Trt)-OPfp (150.4 mg, 0.2 mmole) and HOBt (27.0 mg, 0.2
mmole) (60 min), 2) Fmoc-Gly-OPfp (92.7 mg, 0.2 mmole) and HOBt (27.0 mg,
25 0.2 mmole) (60 min), 3) Fmoc-Trp(Boc)-OH (105.3 mg, 0.2 mmole), HATU (76.0
mg, 0.2 mmole) and N,N-diisopropylethylamine (0.17 ml, 1.0 mmole) (60 min),
4) Fmoc-Arg(Pbf)-OH (129.8 mg, 0.2 mmole), HATU (76.0 mg, 0.2 mmole) and
N,N-diisopropylethylamine (0.17 ml, 1.0 mmole) (60 min), 5) Fmoc-D-Bpa-OH
(98.3 mg, 0.2 mmole), HATU (76.0 mg, 0.2 mmole) and N,N-diisopropyl-
30 ethylamine (0.17 ml, 1.0 mmole) (60 min), 6) Fmoc-His(Trt)-OH (123.9 mg, 0.2
mmole), HATU (76.0 mg, 0.2 mmole) and N,N-diisopropylethylamine (0.17 ml,
1.0 mmole) (60 min), 7) Fmoc-Glu(OBut)-OPfp (118.3 mg, 0.2 mmole) and
HOAt (27.2 mg, 0.2 mmole) (30 min), 8) Fmoc-Cys(Trt)-OPfp (150.4 mg, 0.2
mmole) and HOBt (27.0 mg, 0.2 mmole) (60 min).

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Release and deprotection. A procedure essentially similar to that of Example 1 was used, the yield for HS006 being 37.7 mg. HPLC data (2 x 250 mm column, Vydac RP C18, 90A, 201HS1010): k' (main substance) = 4.89 (a gradient in 30 minutes from 18 % MeCN to 60 % MeCN in 0.1 % trifluoroacetic acid).

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Formation of disulphide. Intramolecular disulphide bridges were formed by an identical approach as in Example 1. The product was HPLC purified also using the same approach as in Example 1. Fractions containing the main peak were pooled and lyophilized. A white powder formed. Yield 11.3 mg (17 %). R_f 0.49 (1-butanol - pyridine - acetic acid - water, 4:1:1:2). Mass spectrometry data: m/e = 1080.3.

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Example 3. *Synthesis of cyclo(S-S)-[Ac-Cys⁴, Arg⁵, D-Nal⁷, Cys-NH₂¹¹] α -MSH(4-11)ditrifluoroacetate (HS007).* The same approach as in Example 1 was used, the starting procedure being identical.

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Amino acid coupling. Eight amino acid attachment cycles were then applied using the approach of Example 1; the reagents and treatment times being (in order) 1) Fmoc-Cys(Trt)-OPfp (150.4 mg, 0.2 mmole) and HOBt (27.0 mg, 0.2 mmole) (60 min), 2) Fmoc-Gly-OPfp (92.7 mg, 0.2 mmole) and HOBt (27.0 mg, 0.2 mmole) (60 min), 3) Fmoc-Trp(Boc)-OH (105.3 mg, 0.2 mmole), HATU (76.0 mg, 0.2 mmole) and N,N-diisopropylethylamine (0.17 ml, 1.0 mmole) (60 min), 4) Fmoc-Arg(Pbf)-OH (129.8 mg, 0.2 mmole), HATU (76.0 mg, 0.2 mmole) and N,N-diisopropylethylamine (0.17 ml, 1.0 mmole) (60 min), 5) Fmoc-D-Nal-OH (78.7 mg, 0.2 mmole), HATU (76.0 mg, 0.2 mmole) and N,N-diisopropylethylamine (0.17 ml, 1.0 mmole) (60 min), 6) Fmoc-His(Trt)-OH (123.9 mg, 0.2 mmole), HATU (76.0 mg, 0.2 mmole) and N,N-diisopropylethylamine (0.17 ml, 1.0 mmole) (60 min), 7) Fmoc-Arg(Pbf)-OH (129.8 mg, 0.2 mmole), HATU (76.0 mg, 0.2 mmole) and N,N-diisopropylethylamine (0.17 ml, 1.0 mmole) (60 min), 8) Fmoc-Cys(Trt)-OPfp (150.4 mg, 0.2 mmole) and HOAt (27.2 mg, 0.2 mmole) (60 min).

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Release and deprotection. The procedure used was essentially identical to that in Example 1, the yield for HS007 being 37.1 mg. HPLC data (2 x 250 mm column, Vydac RP C18, 90A, 201HS1010): k' (main substance) = 3.75 (a

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gradient in 30 minutes from 18% MeCN to 60% MeCN in 0.1% trifluoroacetic acid).

- Formation of disulphide.* An intramolecular disulphide bond was formed by an approach identical to Example 1. The product was purified by HPLC using essentially the same approach as described in Example 1 (10 x 250 mm, Vydac RP C18, 90A, 201HS1010), eluate - 27 % acetonitrile in water + 0.1% trifluoroacetic acid, detection at 290 nm). Fractions, containing the main peak, were pooled and lyophilized. A white powder formed. Yield 7.6 mg (11 %). R_f 0.49 (1-butanol - pyridine - acetic acid - water, 4:1:1:2). Mass spectrometry data: m/e = 1153.4.

- Example 4.** *Synthesis of cyclo(S-S)-(Ac-Cys³, L-Nle⁴, D-Nal⁷, Cys-NH₂¹¹) α -MSH(3-11)trifluoroacetate (HS010).* The same approach as in Example 1 was used, the starting procedure being exactly identical.

- Amino acid coupling.* Repeated aminoacid attachment cycles were then performed, each cycle consisting of 30 - 60 min circulation of appropriate reagents (as detailed below) dissolved in 4 ml DMF through the column, followed by washing with DMF, treatment with 0.3 M acetic anhydride in DMF for 5 min (unless not otherwise specified), and again washing with DMF before the start of the next cycle. sing this procedure 9 cycles were applied the reagents and treatment times being (in order): 1) Fmoc-Cys(Trt)-OPfp (150.4 mg, 0.2 mmole) and HOBt (27.0 mg, 0.2 mmole) (30 min), 2) Fmoc-Gly-OPfp (92.7 mg, 0.2 mmole) and HOBt (27.0 mg, 0.2 mmole) (30 min), 3) Fmoc-Trp(Boc)-OH (105.3 mg, 0.2 mmole), HATU (76.0 mg, 0.2 mmole) and N,N-diisopropylethylamine (0.17 ml, 1.0 mmole) (30 min), 4) Fmoc-Arg(Pbf)-OH (129.8 mg, 0.2 mmole), HATU (76.0 mg, 0.2 mmole) and N,N-diisopropylethylamine (0.17 ml, 1.0 mmole) (60 min), 5) Fmoc-D-Nal-OH (78.7 mg, 0.2 mmole), HATU (76.0 mg, 0.2 mmole) and N,N-diisopropylethylamine (0.17 ml, 1.0 mmole) (60 min), 6) Fmoc-His(Trt)-OH (123.9 mg, 0.2 mmole), HATU (76.0 mg, 0.2 mmole) and N,N-diisopropylethylamine (0.17 ml, 1.0 mmole) (60 min), 7) Fmoc-Glu(Obut)-OPfp (118.3 mg, 0.2 mmole) and HOBt(27.0 mg, 0.2 mmole) (30 min), 8) Fmoc-Nle-OPfp (103.9 mg, 0.2 mmole) and HOBt(27.2 mg, 0.2 mmole) (30 min), 9) Fmoc-Cys(Trt)-OPfp (150.4 mg, 0.2 mmole) and HOBt

(27.0 mg, 0.2 mmole) (30 min). After the last coupling cycle the column was treated for 5 min with 20% piperidine in DMF, and washed again.

Release and deprotection. The procedure used was essentially identical with that of Example 1, the yield for HS010 being 44.1 mg. HPLC data (2 x 250 mm column, Vydac RP C18, 90A, 201HS1010): k' (main substance) = 5.0 (a gradient in 30 minutes from 18% MeCN to 60% MeCN in 0.1% trifluoroacetic acid).

Formation of disulphide. Intramolecular disulphide bridges were formed by an approach essentially identical with that of Example 1. The product was purified by HPLC also using essentially the same approach as in Example 1 (10 x 250 mm, Vydac RP C18, 90A, 201HS1010), eluate - 27 % acetonitrile in water + 0.1% trifluoroacetic acid, detection at 290 nm). Fractions, containing the main peak, were pooled and lyophilized. A white powder formed. Yield 11.5 mg (17 %). R_f 0.64 (1-butanol - pyridine - acetic acid - water, 4:1:1:2). Mass spectrometry data: m/e = 1240.9.

Example 5. *Synthesis of cyclo(S-S)-[Ac-Cys⁴, D-Phe⁷, Cys-NH₂¹¹] α -MSH(4-11)trifluoroacetate (HS963).* The peptide was synthesized using Fmoc based chemistry by the approach essentially as described in Example 1, the intramolecular disulphide bond being formed by heating of the solution of the raw peptide in dimethylsulfoxide using the method described in Example 1. The cyclic peptide was purified by HPLC and fractions, containing the main peak, were pooled and lyophilized. A white powder formed. Yield 14.6 mg (24 %). R_f 0.38 (1-butanol - pyridine - acetic acid - water, 4:1:1:2). K' =0.28 (24% MeCN in 0.1 % trifluoroacetic acid). Mass-spectrometry data: m/e = 1078.2.

Example 6. *Synthesis of cyclo(S-S)-[Ac-Cys⁴, D-Nal⁷, Cys-NH₂¹¹] α -MSH(4-11)trifluoroacetate (HS964).* The peptide was synthesized using Fmoc based chemistry by the approach essentially as described in Example 1, the intramolecular disulphide bond being formed by heating of the solution of the raw peptides in dimethylsulfoxide, using the method described in Example 1. The cyclic peptide was purified by HPLC and fractions, containing the main peak, were pooled and lyophilized. A white powder formed. Yield 17.5 mg (28

5 %). R_f 0.41 (1-butanol - pyridine - acetic acid - water, 4:1:1:2). $K' = 2.14$ (24 % MeCN in 0.1 % trifluoroacetic acid). Mass-spectrometry data: $m/e = 1129.3$.

Example 7. Synthesis of cyclo(S-S)-(Ac-L-Cys⁴, L-Ala⁶, D-Nal⁷, L-Cys¹¹-NH₂)

- 5 **α -MSH₄₋₁₁ (HS011).** The peptide was synthesized on the 0.02 mmole scale using Fmoc based chemistry by the approach essentially as described in Example 1, with the intramolecular disulphide bonds being formed by heating solutions of the peptides in dimethylsulfoxide using the method described in Example 1. The cyclic peptide was purified by HPLC and fractions, containing
10 the main peak, were pooled and lyophilized. White powder; yield 14.2 mg (67 %). R_f 0.66 (1-butanol-pyridine-acetic acid-water, 4:1:1:2). $k' = 6.0$ (a gradient in 30 minutes from 18 % MeCN to 60 % MeCN in 0.1% trifluoroacetic acid). Mass spectrometry data: $m/e = 1061.3$.

15 **Example 8. Synthesis of cyclo(S-S)-(Ac-L-Nle³, L-Cys⁴, D-Nal⁷, L-Cys¹¹-NH₂)**

- α -MSH₄₋₁₁ (HS012).** The peptide was synthesized on the 0.02 mmole scale using Fmoc based chemistry by the approach essentially as described in Example 1, with the intramolecular disulphide bond being formed by heating solutions of the peptide in dimethylsulfoxide using the method described in
20 Example 1. The cyclic peptide was purified by HPLC and fractions, containing the main peak, were pooled and lyophilized. White powder; yield 11.0 mg (44 %). R_f 0.62 (1-butanol-pyridine-acetic acid-water, 4:1:1:2). $k' = 5.2$ (a gradient in 30 minutes from 18 % MeCN to 60 % MeCN in 0.1% trifluoroacetic acid). Mass spectrometry data: $m/e = 1239.2$.

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Example 9. Synthesis of cyclo(S-S)-(Ac-L-Cys¹¹, D-Nal¹⁴, L-Cys¹⁸,

- L-Asp-NH₂²²) β -MSH₁₁₋₂₂ trifluoroacetate (HS014).** The peptide was synthesized on the 0.02 mmole scale using Fmoc based chemistry by the approach essentially as described in Example 1 with the intramolecular
30 disulphide bond being formed by heating solutions of the peptide in dimethylsulfoxide using the method described in Example 1. The cyclic peptide was purified by HPLC and fractions, containing the main peak, were pooled and lyophilized. A white powder formed. Yield 8.6 mg (27 %). R_f 0.35 (1-butanol-pyridine-acetic acid-water, 4:1:1:2). $k' = 4.0$ (a gradient in 30

minutes from 18 % MeCN to 60 % MeCN in 0.1% trifluoroacetic acid). Mass spectrometry data: m/e 1564.1.

Example 10. Assay of binding affinities of peptides for human MC-receptors.

- 5 Expression of receptor clones. Human MC1- and MC5-receptor DNAs (Chhajlani and Wikberg 1992; Chhajlani et al., 1993), cloned into the expression vector pRc/CMV (InVitrogen Corp., USA), and human MC3- and human MC4-receptor DNAs (Gantz et al., 1993a & b), cloned into the expression vector pCMV/neo, were used. COS cells were grown and
- 10 transfected with receptor clones as described (Schiöth et al. 1995, 1996b). After transfection cells were cultivated for 48 h, detached from the petri dishes, and used for radioligand binding as described (Schiöth et al. 1995, 1996b).

- Binding studies. The transfected cells were washed with binding buffer
- 15 (Minimum Essential Medium with Earle's salts, 25 mM HEPES, pH 7.0, 0.2 % bovine serum albumin, 1 mM 1,10-phenanthroline, 0.5 mg per litre leupeptin and 200 mg per litre bacitracin) and distributed into 96 well plates. The cells were then incubated for 2 h at 37°C, with 0.1 ml binding buffer in each well containing [¹²⁵I][Nle⁴, D-Phe⁷]α-MSH and appropriate concentrations of the
- 20 peptide to be tested. After incubation the plates were put on ice and the cells was washed with 0.1 ml of ice-cold binding buffer. The cells were then detached from the plates with 0.2 ml of 0.1 N NaOH. Radioactivity was counted by using a Wallac Wizard automatic gamma counter. The competition data were analysed by fitting it to the logistic function using non-linear regression
- 25 analysis. The K_i-values were then calculated from the thus obtained IC₅₀-values by using the Cheng and Prusoff equation. (Biochem. Pharmacol. 1973, 22, 3099.)

- Example 11. Synthesis of cyclo(S-S)-(Ac-L-Cys⁴, D-Nal⁷, L-Asp¹⁰, L-Cys-NH₂¹¹) α-MSH₄₋₁₁ (HS009) was made essentially as described in Example 1.**
- 30 1. Yield 28.7%. R_f 0.55. k' 5.50(21% MeCN in 0.1% TFA). m/e 1185.

- Example 12. Synthesis of cyclo(S-S)-(Ac-L-Cys⁴, L-Ala⁶, D-Nal⁷, L-Cys-NH₂¹¹) α-MSH₄₋₁₁ (HS011) was made essentially as described in Example 1.**
- 35 Yield 34.4%. R_f 0.70. k' 1.68 (27% MeCN in 0.1% TFA). m/e 1061.

Example 13. Synthesis of cyclo(S-S)-(Ac-L-Cys⁴, L-Pro⁶, D-Nal⁷, L-Cys-NH₂¹¹) α-MSH₄₋₁₁ (HS015) was made essentially as described in Example 1. Yield 21.2%. R_f 0.70. k' 7.50 (25.2% MeCN in 0.1% TFA). m/e 1086.

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Example 14. Synthesis of cyclo(S-S)-(Ac-L-Cys⁴, L-Arg⁵, D-Nal⁷, L-Cys¹¹, L-Asp¹², L-Arg-L-Phe-NH₂¹³) α-MSH₄₋₁₃ (HS016) was made essentially as described in Example 1. Yield 18.0%. R_f 0.50. k' 3.08 (19.8% MeCN in 0.1% TFA). m/e 1572.

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Example 15. Synthesis of cyclo(S-S)-(Ac-L-Cys⁴, L-Glu⁶, D-Nal⁷, L-Cys-NH₂¹¹) α-MSH₄₋₁₁ (HS017) was made essentially as described in Example 1. Yield 23.2%. R_f 0.59. k' 6.31 (22.8% MeCN in 0.1% TFA). m/e 1119.

15 **Example 16.** Synthesis of cyclo(S-S)-(Ac-L-Cys³, L-Glu⁴, L-His⁵, Gly⁶, D-Nal⁷, L-Cys-NH₂¹⁰) α-MSH₄₋₁₁ (HS018) was made essentially as described in Example 1. Yield 17.3%. R_f 0.53. k' 3.67 (19.8% MeCN in 0.1% TFA). m/e 1127.

Example 17. Synthesis of cyclo(S-S)-(Ac-L-Pro⁸, L-Cys¹¹, D-Nal¹⁴, L-Cys¹⁸, L-Asp-NH₂²²) β-MSH₈₋₂₂ (HS019) was made essentially as described in Example 1. Yield 12.0%. R_f 0.55. k' 0.95 (18% MeCN in 0.1% TFA). m/e 1981.

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Example 18. Synthesis of cyclo(S-S)-(Ac-L-Tyr¹, L-Val², L-Cys³, L-Nle⁴, D-Nal⁷, L-Cys¹¹, L-Asp¹², L-Arg-L-Phe-NH₂¹³) α-MSH₁₋₁₃ (HS020) was made essentially as described in Example 1. Yield 14.5%. R_f 0.64. k' 3.84 (25.8% MeCN in 0.1% TFA). m/e 1922.

25

Example 19. Synthesis of cyclo(S-S)-(Ac-L-Cys⁴, Gly⁶, D-Nal⁷, L-Cys-NH₂¹¹) α-MSH₄₋₁₁ (HS023) was made essentially as described in Example 1. Yield 22.0%. R_f 0.66. k' 5.00 (20.4% MeCN in 0.1% TFA). m/e 1047.

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Example 20. Synthesis of cyclo(S-S)-(Ac-L-Cys³, L-Nle⁴, L-Arg⁵, D-Nal⁷, L-Cys-NH₂¹¹) α-MSH₄₋₁₁ (HS024) was made essentially as described in Example 1. Yield 20.1%. R_f 0.63. k' 4.95 (20.4% MeCN in 0.1% TFA). m/e 1268.

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Example 21. Synthesis of cyclo(S-S)-(Ac-L-Cys¹¹, D-3,4-dichlorophenyl-alanine¹⁴, L-Cys¹⁸, L-Asp-NH₂²²) β-MSH₁₁₋₂₂ (HS028) was made essentially as described in Example 1. Yield 10.5 %. R_f 0.38. k' 1.22(17.4% MeCN in 0.1% TFA). m/e 1582.

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Example 22. Synthesis of cyclo(S-S)-(Ac-L-Cys¹¹, D-4-fluorophenylalanine¹⁴, L-Cys¹⁸, L-Asp-NH₂²²) β-MSH₁₁₋₂₂ (HS029) was made essentially as described in Example 1. Yield 7.1%. R_f 0.37. k' 0.22(15% MeCN in 0.1% TFA). m/e 1532.

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Example 23. Synthesis of cyclo(S-S)-(Ac-L-Cys¹¹, D-4-nitrophenylalanine¹⁴, L-Cys¹⁸, L-Asp-NH₂²²) β-MSH₁₁₋₂₂ (HS030) was made essentially as described in Example 1. Yield 7.6 %. R_f 0.40. k' 0.53(13.8 % MeCN in 0.1% TFA). m/e 1580.

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Example 24. Synthesis of cyclo(S-S)-(Ac-L-Pro⁸, L-Cys¹⁰, L-Nle¹¹, L-Arg¹², D-Nal¹⁴, L-Cys¹⁸, L-Asp-NH₂²²) β-MSH₈₋₂₂ (HS031) was made essentially as described in Example 1. Yield 6.7 %. R_f 0.44. k' 0.89(20.4% MeCN in 0.1% TFA). m/e 1964.

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Example 25. Synthesis of cyclo(S-S)-(Ac-L-Cys¹⁰, L-Nle¹¹, L-Arg¹², D-Nal¹⁴, L-Cys¹⁸, L-Asp-NH₂²²) β-MSH₁₀₋₂₂ (HS032) was made essentially as described in Example 1. Yield 15.0%). R_f 0.43. k' 0.61(18% MeCN in 0.1% TFA). m/e 1704.

25

Example 26. Synthesis of cyclo(S-S)-(Ac-L-Cys³, L-Nle⁴, D-Nal⁷, L-Cys¹⁰) α-MSH₃₋₁₃ (HS040) was made essentially as described in Example 1. Yield 32.3 %. R_f 0.57. k' 1.0(18% MeCN in 0.1% TFA). m/e 1506.

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Example 27. Synthesis of cyclo(S-S)-(Ac-L-Cys³, L-Nle⁴, L-Arg⁵, D-Nal⁷, L-Cys-NH₂¹¹) α-MSH₁₋₁₁ (HS050) was made essentially as described in Example 1. Yield 15.3 %. R_f 0.69. k' 1.44(18.6% MeCN in 0.1% TFA). m/e 1516.

Example 28. Synthesis of cyclo(S-S)-(Ac-L-Cys³, L-Nle⁴, D-Nal⁷, L-Cys-NH₂¹¹) α -MSH₁₋₁₁ (HS051) was made essentially as described in Example 1. Yield 13.3 %. R_f 0.70. k' 2.61 (22.8% MeCN in 0.1% TFA). m/e 1489.

5 **Example 29.** Synthesis of cyclo(S-S)-(Ac-L-Cys³, L-Nle⁴, L-Arg⁵, D-Nal⁷, L-Cys-NH₂¹¹) α -MSH₃₋₁₁ (HS052) was made essentially as described in Example 1. Yield 30.0 %. R_f 0.70. k' 0.83(22.8% MeCN in 0.1% TFA). m/e 1258.

10 **Example 30.** Synthesis of cyclo(S-S)-(Ac-L-Cys³, L-Nle⁴, L-Lys⁵, D-Nal⁷, L-Cys-NH₂¹¹) α -MSH₃₋₁₁ (HS053) was made essentially as described in Example 1. Yield 47.6%. R_f 0.62. k' 0.44(19.2% MeCN in 0.1% TFA). m/e 1238.

15 **Example 31.** Synthesis of cyclo(S-S)-(Ac-L-Cys³, L-Phe⁴, L-Arg⁵, D-Nal⁷, L-Cys-NH₂¹¹) α -MSH₃₋₁₁ (HS054) was made essentially as described in Example 1. Yield 23.4 %. R_f 0.66. k' 3.00(15.6% MeCN in 0.1% TFA). m/e 1300.

20 **Example 32.** Synthesis of cyclo(S-S)-(Ac-L-Cys³, L-Asn⁴, L-Arg⁵, D-Nal⁷, L-Cys-NH₂¹¹) α -MSH₃₋₁₁ (HS055) was made essentially as described in Example 1. Yield 29.6%. R_f 0.54. k' 0.44(13.8% MeCN in 0.1% TFA). m/e 1267.

25 **Example 33.** Synthesis of cyclo(S-S)-(Ac-L-Cys³, L-Nle⁴, L-Arg⁵, D-Nal⁷, L-Cys¹¹, L-Tyr-NH₂¹²) α -MSH₃₋₁₂ (HS058) was made essentially as described in Example 1. Yield 22.5%. R_f 0.67. k' 2.57(19.2% MeCN in 0.1% TFA). m/e 1429.

30 **Example 34.** Synthesis of cyclo(S-S)-(Ac-L-Cys³, L-Nle⁴, L-Arg⁵, D-Nal⁷, L-Tyr¹⁰, L-Cys-NH₂¹¹) α -MSH₃₋₁₁ (HS059) was made essentially as described in Example 1. Yield 20%. R_f 0.65. k' 2.83(21.6% MeCN in 0.1% TFA). m/e 1372.

Example 35. Synthesis of cyclo(S-S)-(Ac-L-Cys³, L-Nle⁴, L-Arg⁵, D-3,4-dichlorophenylalanine⁷, L-Cys-NH₂¹¹) α -MSH₃₋₁₁ (HS060) was made

essentially as described in Example 1. Yield 30.3%. R_f 0.59. k' 2.50(18.0% MeCN in 0.1% TFA). m/e 1285.

5 **Example 36.** Synthesis of cyclo(S-S)-(Ac-L-Cys³, L-Nle⁴, L-Arg⁵, D-4-fluorophenylalanine⁷, L-Cys-NH₂¹¹) α-MSH₃₋₁₁ (HS061) was made essentially as described in Example 1. Yield 21.6%. R_f 0.58. k' 2.23(19.2% MeCN in 0.1% TFA). m/e 1234.

10 **Example 37.** Synthesis of cyclo(S-S)-(Ac-L-Cys³, L-Nle⁴, L-Arg⁵, D-4-nitrophenylalanine⁷, L-Cys-NH₂¹¹) α-MSH₃₋₁₁ (HS062) was made essentially as described in Example 1. Yield 36.0%. R_f 0.58. k' 2.11(19.2% MeCN in 0.1% TFA). m/e 1261.

15 **Example 38.** Synthesis of cyclo(S-S)-(Ac-L-Cys³, L-Nle⁴, L-Arg⁵, D-Cha⁷, L-Cys-NH₂¹¹) α-MSH₃₋₁₁ (HS063) was made essentially as described in Example 1. Yield 27.8%. R_f 0.59. k' 2.11(21.6% MeCN in 0.1% TFA). m/e 1222.

20 **Example 39.** Synthesis of cyclo(S-S)-(Ac-L-Cys³, L-Nle⁴, L-Arg⁵, D-Bpa⁷, L-Cys-NH₂¹¹) α-MSH₃₋₁₁ (HS064) was made essentially as described in Example 1. Yield 21.2%. R_f 0.60. k' 2.55(21.6% MeCN in 0.1% TFA). m/e 1320.

Example 40. Synthesis of cyclo(S-S)-(Ac-L-Cys³, L-Nle⁴, L-Arg⁵, D-L-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid⁷, L-Cys-NH₂¹¹) α-MSH₃₋₁₁ (HS069) was made essentially as described in Example 1. Yield 20.8%. R_f 0.57. k' 1.83(16.8% MeCN in 0.1% TFA). m/e 1228.

30 **Example 41.** Synthesis of cyclo(S-S)-(Ac-L-Cys¹¹, D-L-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid¹⁴, L-Cys¹⁸, L-Asp-NH₂²²) β-MSH₁₁₋₂₂ (HS071) was made essentially as described in Example 1. Yield 8.2%. R_f 0.30. k' 1.33(13.8% MeCN in 0.1% TFA). m/e 1525.

Example 42. Synthesis of cyclo(S-S)-(Ac-L-Cys¹⁰, L-Nle¹¹, L-Arg¹², D-Nal¹⁴, L-Cys¹⁸, L-Tyr-NH₂²²) β-MSH₁₁₋₂₂ (HS072) was made essentially as described in Example 1. Yield 19.1%. R_f 0.48. k' 3.25(18% MeCN in 0.1% TFA). m/e 1752.

Example 43. Synthesis of cyclo(S-S)-(Ac-L-Cys¹¹, D-3-benzothienylalanine¹⁴, L-Cys¹⁸, L-Asp-NH₂²²) β-MSH₁₁₋₂₂ (HS073) was made essentially as described in Example 1. Yield 13.7%. R_f 0.35. k' 0.89(16.8% MeCN in 0.1%
5 TFA). m/e 1569.

Example 44. Synthesis of cyclo(S-S)-(Ac-L-Cys¹¹, D-4-cyanophenylalanine¹⁴, L-Cys¹⁸, L-Asp-NH₂²²) β-MSH₁₁₋₂₂ (HS074) was made essentially as described in Example 1. Yield 9.1%. R_f 0.38. k' 1.00(13.8% MeCN in 0.1%
10 TFA). m/e 1538.

Example 45. Synthesis of cyclo(S-S)-(Ac-L-Cys¹¹, D-4-iodophenylalanine¹⁴, L-Cys¹⁸, L-Asp-NH₂²²) β-MSH₁₁₋₂₂ (HS078) was made essentially as described in Example 1. Yield 10.7%. R_f 0.40. k' 1.67(16.2% MeCN in 0.1%
15 TFA). m/e 1639.

Example 46. Synthesis of cyclo(S-S)-(Ac-L-Cys¹¹, D-4-bromophenylalanine¹⁴, L-Cys¹⁸, L-Asp-NH₂²²) β-MSH₁₁₋₂₂ (HS082) was made essentially as described in Example 1. Yield 10.7%. R_f 0.40. k' 1.67(16.2% MeCN in 0.1%
20 TFA). m/e 1639.

Example 47. Synthesis of cyclo(S-S)-(Ac-L-Cys¹¹, D-4,4'-biphenylalanine¹⁴, L-Cys¹⁸, L-Asp-NH₂²²) β-MSH₁₁₋₂₂ (HS083) was made essentially as described in Example 1. Yield 17.0%. R_f 0.39. k' 1.94(17.4% MeCN in 0.1%
25 TFA). m/e 1589.

Example 48. Synthesis of cyclo(S-S)-(Ac-L-Cys¹¹, D-pentafluorophenylalanine¹⁴, L-Cys¹⁸, L-Asp-NH₂²²) β-MSH₁₁₋₂₂ (HS084) was made essentially as described in Example 1. Yield 14.6%. R_f 0.38. k' 0.89(17.4%
30 MeCN in 0.1% TFA). m/e 1603.

Example 49. Synthesis of cyclo(S-S)-(Ac-L-Cys¹¹, D-β,β-diphenylalanine¹⁴, L-Cys¹⁸, L-Asp-NH₂²²) β-MSH₁₁₋₂₂ (HS085) was made essentially as described in Example 1. Yield 11.0%. R_f 0.37. k' 1.28(15% MeCN in 0.1%
35 TFA). m/e 1589.

Example 50. Synthesis of cyclo(S-S)-(Ac-L-Cys³, L-Nle⁴, D-benzothienyl-alanine⁷, L-Cys-NH₂¹¹) α-MSH₃₋₁₁ (HS086) was made essentially as described in Example 1. Yield 35.3%. R_f 0.64. k' 3.77(22.2% MeCN in 0.1%
5 TFA). m/e 1245.

Example 51. Synthesis of cyclo(S-S)-(Ac-L-Cys³, L-Nle⁴, D-4-cyanophenyl-alanine⁷, L-Cys-NH₂¹¹) α-MSH₃₋₁₁ (HS087) was made essentially as described in Example 1. Yield 28.8%. R_f 0.60. k' 3.22(19.8% MeCN in 0.1%
10 TFA). m/e 1214.

Example 52. Synthesis of cyclo(S-S)-(Ac-L-Cys³, L-Nle⁴, D-4-iodophenyl-alanine⁷, L-Cys-NH₂¹¹) α-MSH₃₋₁₁ (HS091) was made essentially as described in Example 1. Yield 20.5%. R_f 0.65. k' 3.61(22.8% MeCN in 0.1%
15 TFA). m/e 1315.

Example 53. Synthesis of cyclo(S-S)-(Ac-L-Cys³, L-Nle⁴, D-4-bromophenyl-alanine⁷, L-Cys-NH₂¹¹) α-MSH₃₋₁₁ (HS095) was made essentially as described in Example 1. Yield 30.3%. R_f 0.64. k' 2.61(22.2% MeCN in 0.1%
20 TFA). m/e 1268.

Example 54. Synthesis of cyclo(S-S)-(Ac-L-Cys³, L-Nle⁴, D-4,4'-biphenyl-alanine⁷, L-Cys-NH₂¹¹) α-MSH₃₋₁₁ (HS096) was made essentially as described in Example 1. Yield 23.3%. R_f 0.66. k' 2.94(25.2% MeCN in 0.1%
25 TFA). m/e 1265.

Example 55. Synthesis of cyclo(S-S)-(Ac-L-Cys³, L-Nle⁴, D-pentafluorophenyl-alanine⁷, L-Cys-NH₂¹¹) α-MSH₃₋₁₁ (HS097) was made essentially as described in Example 1. Yield 29.3%. R_f 0.64. k' 2.61(22.8% MeCN in 0.1%
30 TFA). m/e 1269.

Example 56. Synthesis of cyclo(S-S)-(Ac-L-Cys³, L-Nle⁴, D-β,β-diphenyl-alanine⁷, L-Cys-NH₂¹¹) α-MSH₃₋₁₁ (HS098) was made essentially as described in Example 1. Yield 27.6%. R_f 0.66. k' 2.66(21% MeCN in 0.1%
35 TFA). m/e 1265.

Example 57. Increase of food intake by HS014.

In order to demonstrate the capacity of HS014 to increase food intake male Wistar rats weighing 290-320g) were anaesthetised with chloral hydrate (350 mg/kg i.p.) and fixed in a stereotaxic frame. Stainless steel guide cannulae were implanted just above the left lateral ventricle of each rat. The cannula was lowered until its end was 3.2 mm below the skull and fixed to the bone with stainless steel screws and dental acrylic cement and closed with stylets when not in use. Rats were frequently handled and weighed during the recovery period and habituated to the introduction of Petri dishes with food into their home cages which were used in measurement of food intake. Two days before the experiment the rats were sham injected to test the correct placement of the cannulae. HS014 was dissolved in saline to provide the concentration of 2 nmol/microlitre. The feeding experiments were performed starting from 7th day after the surgery. On the day of the experiment the food was removed from wire baskets and the rats were injected intracerebroventricularly with saline or HS014 (0.1-10 nmol) over 1 min using a 33 gauge injector connected to the 50 microlitre Hamilton syringe and an infusion pump (World Precision Instruments, Sarasota, USA). The movement of an air bubble inside the PE20 polyethylene tubing confirmed the drug flow. The needle was left in place for 30 seconds, then removed and the cannula closed with stylet and the rat returned to its home cage. All injections were carried out between 12.00-13.00 every third day and were given in randomised order. Food intake was then measured after 0.5, 1, 2 and 4 h following the intracerebroventricular injection.

25

Results are shown in Fig. 1. As can be seen administration of 0.1 - 10 nmol HS014 induced significant increases in food intake during 0 - 4 hrs, both when measured during divided periods (top panel of Fig. 1) and when estimated as cumulative food intake during the extent of the experiment (bottom panel of Fig. 1). In Fig.1 * denotes a significant difference compared to the vehicle control at $p > 0.05$. ** denotes a significance level of $p < 0.01$.

30

Example 58. Increase of rate of growth by HS028

To demonstrate the capacity of HS028 to increase body weight and rate of growth rats were operated upon under brietal (3%, 0.2 ml./100g) anaesthesia in order to implant a brain infusion kit connected to an osmotic minipump (Alzet, 2001) into the right lateral cerebral ventricle - 1.0 mm posterior, 1.5 mm lateral - to the bregma, for intracerebroventricular infusion. Dental glas ionophor and a screw were used to secure the infusion kit in position. The osmotic minipumps were placed subcutaneously in the midscapular region of the back. After 10 days of drug administration the osmotic minipumps were removed under light brietal anaesthesia.

The results are shown in Fig 2. As can be seen rats treated with 0.07 - 0.7 micromol HS028/hr increased their body weight much faster during the 10 days treatment period (marked "injection" in Fig. 2) compared to the control animals (sham operated). After termination of infusion of HS028 (marked "recovery" in Fig. 2) the rate of growth declined and became similar to the sham operated controls.

20 FIGURES

Fig. 1a Effect of administration of a single intracerebroventricular dose of between 0.1 and 10 nmol of HSO14 on food intake in free-feeding rats: data shown for each period of measurement;

25

Fig. 1b Effect of administration of a single intracerebroventricular dose of between 0.1 and 10 nmol of HSO14 on cumulative food intake in free-feeding rats;

30 Fig. 2 Effect of continuous intracerebroventricular infusion of 0.07 or 0.7 micromol HS028 per hour on body weight (BWt) of rats. The infusion was started on December 17, 1997 and stopped on December 27, 1997. Controls were infused with the vehicle for HS028.

35

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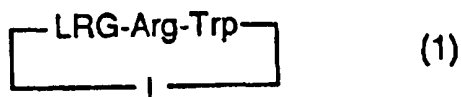
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Claims

1. Cyclic MC-receptor activating and/or blocking peptide of the general formula (1):

5



10 in which LRG is a large aminoacid and L is a linker connecting LRG and Trp and thereby forming a cycle, with the proviso that the cycle has from 26 to 29 members.

2. The peptide of claim 1, wherein L (2) is:

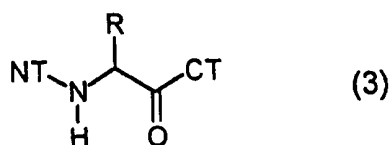
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20 in which X is a non-cyclic peptide of two to three aminoacids, Y is a non-cyclic peptide of one to two aminoacids, A and B are non-cyclic peptides, each of from 0 to 5 aminoacids, and in which the cystein residues are connected by a disulphide bond.

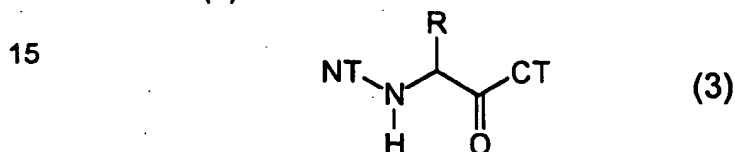
3. The peptide of claim 1 or 2, wherein aminoacid is selected from alanine, arginine, asparagine, aspartic acid, p-benzoyl-phenylalanine, β -cyclohexyl-alanine, cysteine, glutamic acid, glutamine, glycine, histidine, isoleucine, leucine, lysine, methionine, β -(2-naphtyl)-alanine, norleucine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, valine, 3,4-Dichlorophenyl-alanine, 4-Fluorophenylalanine, 4-Nitrophenylalanine, 2-Thienylalanine, 3-Benzothierylalanine, 4-Cyanophenylalanine, 4-Iodophenylalanine, 4-Bromophenylalanine, 4,4'-Biphenylalanine, Pentafluorophenylalanine, β , β -Diphenylalanine in either D- or L-conformations, D-L-1,2,3,4-Tetrahydroisoquinoline-3-carboxylic acid, well as from compounds having the general structure (3):

35



in which R is H or CH₂R¹, wherein R¹ is H, alkyl, substituted alkyl, heteroalkyl, substituted heteroalkyl, alkenyl, substituted alkenyl, heteroalkenyl, substituted heteroalkenyl, alkynyl, substituted alkynyl, heteroalkynyl, substituted heteroalkynyl, cycloalkyl, substituted cycloalkyl, cycloheteroalkyl, substituted cycloheteroalkyl, cycloalkenyl, substituted cycloalkenyl, cycloheteroalkenyl, substituted cycloheteroalkenyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, or functional group, NT being selected from H, functional group and bond to another aminoacid, CT being selected from functional group or bond to another aminoacid, the compounds according to formula (3) being in either D- or L-conformation.

4. The peptide of claims 1-3 where large aminoacid, LRG, has the general structure (3):



20 in which R is H or CH₂R¹ where R¹ is H, alkyl, substituted alkyl, heteroalkyl, substituted heteroalkyl, alkenyl, substituted alkenyl, heteroalkenyl, substituted heteroalkenyl, alkynyl, substituted alkynyl, heteroalkynyl, substituted heteroalkynyl, cycloalkyl, substituted cycloalkyl, cycloheteroalkyl, substituted cycloheteroalkyl, cycloalkenyl, substituted cycloalkenyl, cycloheteroalkenyl, substituted cycloheteroalkenyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, or functional group, NT is H, or functional group or bond to another aminoacid, and CT is functional group or bond to another aminoacid, the substance according to formula (3) being in either D- or L-conformation, wherein R contains at least 14 atoms, preferably at least 15 atoms.

30

5. The peptide of any of claims 1-3, wherein the large aminoacid is selected from the group consisting of D-β-(2-naphtyl)-alanine, D-p-benzoyl-phenylalanine, D-β-cyclohexylalanine, D-3,4-dichlorophenylalanine, D-4-fluorophenylalanine, D-4-nitrophenylalanine, D-3-benzothienylalanine, D-4-cyanophenylalanine, D-4-iodophenylalanine, D-4-bromophenylalanine,

35

D-4,4'-biphenylalanine, D-pentafluorophenylalanine, D-β, β-diphenylalanine and D-L-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid.

6. The D-form of the peptide of claim 1, 2, 3 or 4.

5

7. Any of the following compounds:

HS005 Ac-Cys-Glu-His-D-Cha-Arg-Trp-Gly-Cys-NH₂

HS006 Ac-Cys-Glu-His-D-Bpa-Arg-Trp-Gly-Cys-NH₂

HS007 Ac-Cys-Arg-His-D-Nal-Arg-Trp-Gly-Cys-NH₂

HS010 Ac-Cys-Nle-Glu-His-D-Nal-Arg-Trp-Gly-Cys-NH₂

HS011 Ac-Cys-Glu-Ala-D-Nal-Arg-Trp-Gly-Cys-NH₂

HS012 Ac-Nle-Cys-Glu-His-D-Nal-Arg-Trp-Gly-Cys-NH₂

HS014 Ac-Cys-Glu-His-D-Nal-Arg-Trp-Gly-Cys-Pro-Pro-Lys-Asp-NH₂

25

HS964 Ac-Cys-Glu-His-D-Nal-Arg-Trp-Gly-Cys-NH₂

HS009 Ac-Cys-Glu-His-D-Nal-Arg-Trp-Asp-Cys- NH₂

HS011 Ac-Cys-Glu-Ala-D-Nal-Arg-Trp-Gly-Cys- NH₂

HS015 Ac-Cys-Glu-Pro-D-Nal-Arg-Trp-Gly-Cys- NH₂

HS016 Ac-Cys-Arg-His-D-Nal-Arg-Trp-Gly-Cys-Asp-Arg-Phe- NH₂

HS017 Ac-Cys-Glu-Glu-D-Nal-Arg-Trp-Gly-Cys- NH₂

HS018 Ac-Cys-Glu-His-Gly-D-Nal-Arg-Trp-Cys- NH₂

HS019

Ac-Pro-Tyr-Arg-Cys-Glu-His-D-Nal-Arg-Trp-Gly-Cys-Pro-Pro-Lys-Asp- NH₂

HS020

Ac-Tyr-Val-Cys-Nle-Glu-His-D-Nal-Arg-Trp-Gly-Cys-Asp-Arg-Phe- NH₂


HS023 Ac-Cys-Gly-His-D-Nal-Arg-Trp-Gly-Cys- NH₂

HS024 Ac-Cys-Nle-Gly-His-D-Nal-Arg-Trp-Gly-Cys- NH₂

HS028

Ac-Cys-Glu-His-D-3,4-Dcp-Arg-Trp-Gly-Cys-Pro-Pro-Lys-Asp- NH₂


HS029

Ac-Cys-Glu-His-D-4-Fpa-Arg-Trp-Gly-Cys-Pro-Pro-Lys-Asp- NH₂




HS030

Ac-Cys-Glu-His-D-4-Npa-Arg-Trp-Gly-Cys-Pro-Pro-Lys-Asp- NH₂


HS031

Ac-Pro-Tyr-Cys-Nle-Arg-His-D-Nal-Arg-Trp-Gly-Cys-Pro-Pro-Lys-Asp- NH₂


HS032

Ac-Cys-Nle-Arg-His-D-Nal-Arg-Trp-Gly-Cys-Pro-Pro-Lys-Asp- NH₂
HS040 Ac-Cys-Nle-Glu-His-D-Nal-Arg-Trp-Cys-Lys-Pro-Val- NH₂
HS050 Ac-Ser-Tyr-Cys-Nle-Arg-His-D-Nal-Arg-Trp-Gly-Cys- NH₂
HS051 Ac-Ser-Tyr-Cys-Nle-Glu-His-D-Nal-Arg-Trp-Gly-Cys- NH₂
HS052 Ac-Cys-Nle-Arg-Glu-D-Nal-Arg-Trp-Gly-Cys- NH₂
HS053 Ac-Cys-Nle-Lys-His-D-Nal-Arg-Trp-Gly-Cys- NH₂
HS054 Ac-Cys-Phe-Arg-His-D-Nal-Arg-Trp-Gly-Cys- NH₂


HS055 Ac-Cys-Asn-Arg-His-D-Nal-Arg-Trp-Gly-Cys- NH₂



HS058 Ac-Cys-Nle-Arg-His-D-Nal-Arg-Trp-Gly-Cys-Tyr- NH₂



HS059 Ac-Cys-Nle-Arg-His-D-Nal-Arg-Trp-Tyr-Cys- NH₂



HS060 Ac-Cys-Nle-Arg-His-D-3,4-Dcp-Arg-Trp-Gly-Cys- NH₂



HS061 Ac-Cys-Nle-Arg-His-D-4-Fpa-Arg-Trp-Gly-Cys- NH₂



HS062 Ac-Cys-Nle-Arg-His-D-4-Npa-Arg-Trp-Gly-Cys- NH₂



HS063 Ac-Cys-Nle-Arg-His-D-Cha-Arg-Trp-Gly-Cys- NH₂



HS064 Ac-Cys-Nle-Arg-His-D-Bpa-Arg-Trp-Gly-Cys- NH₂



HS069 Ac-Cys-Nle-Arg-His-D-Tic-Arg-Trp-Gly-Cys- NH₂



HS071

Ac-Cys-Glu-His-D-Tic-Arg-Trp-Gly-Cys-Pro-Pro-Lys-Asp- NH₂



HS072

Ac-Cys-Nle-Arg-His-D-Nal-Arg-Trp-Gly-Cys-Pro-Pro-Lys-Tyr- NH₂



HS073

Ac-Cys-Glu-His-D-3-Bal-Arg-Trp-Gly-Cys-Pro-Pro-Lys-Asp- NH₂

HS074

Ac-Cys-Glu-His-D-4-Ypa-Arg-Trp-Gly-Cys-Pro-Pro-Lys-Asp- NH₂

HS078

Ac-Cys-Glu-His-D-4-lph-Arg-Trp-Gly-Cys-Pro-Pro-Lys-Asp- NH₂

HS082

Ac-Cys-Glu-His-D-4-Rpa-Arg-Trp-Gly-Cys-Pro-Pro-Lys-Asp- NH₂

HS083

Ac-Cys-Glu-His-D-Bip-Arg-Trp-Gly-Cys-Pro-Pro-Lys-Asp- NH₂

HS084

Ac-Cys-Glu-His-D-Pfp-Arg-Trp-Gly-Cys-Pro-Pro-Lys-Asp- NH₂

HS085

Ac-Cys-Glu-His-D-Dip-Arg-Trp-Gly-Cys-Pro-Pro-Lys-Asp- NH₂HS086 Ac-Cys-Nle-Glu-His-D-3-Bal-Arg-Trp-Gly-Cys- NH₂HS087 Ac-Cys-Nle-Glu-His-D-4-Ypa-Arg-Trp-Gly-Cys- NH₂HS091 Ac-Cys-Nle-Glu-His-D-4-lph-Arg-Trp-Gly-Cys- NH₂

HS095 Ac-Cys-Nle-Glu-His-D-4-Rpa-Arg-Trp-Gly-Cys- NH₂

HS096 Ac-Cys-Nle-Glu-His-D-4-Bip-Arg-Trp-Gly-Cys- NH₂

HS097 Ac-Cys-Nle-Glu-His-D-Pfp-Arg-Trp-Gly-Cys- NH₂

HS098 Ac-Cys-Nle-Glu-His-D-Dip-Arg-Trp-Gly-Cys- NH₂

8. Administration of a pharmacologically effective amount of a compound according claim 1-7 to an animal including man for treating a condition related to eating, body weight, anorexia, anorexia nervosa, bulimia, anorexia in elderly, cachexia, cancer chemotherapy, anorexia in patients having cancer, cachexia in patients having cancer, motivation, learning, memory, behaviour, inflammation, body temperature, pain perception, blood pressure, heart rate, vascular tone, natriuresis, brain blood flow, nerve growth, placental development, aldosteron synthesis and release, thyroxin release, spermatogenesis, ovarian weight, prolactin and FSH secretion, uterine bleeding in women, sebum and pheromone secretion, blood glucose levels, intrauterine foetal growth, as well as other events related to parturition, and to afford neuroprotection, as well as for increasing rate of growth, in particular during animal breeding.

9. A pharmaceutical composition comprising a pharmacologically effective amount of a compound according to any of claims 1-7 and a pharmaceutically acceptable carrier.

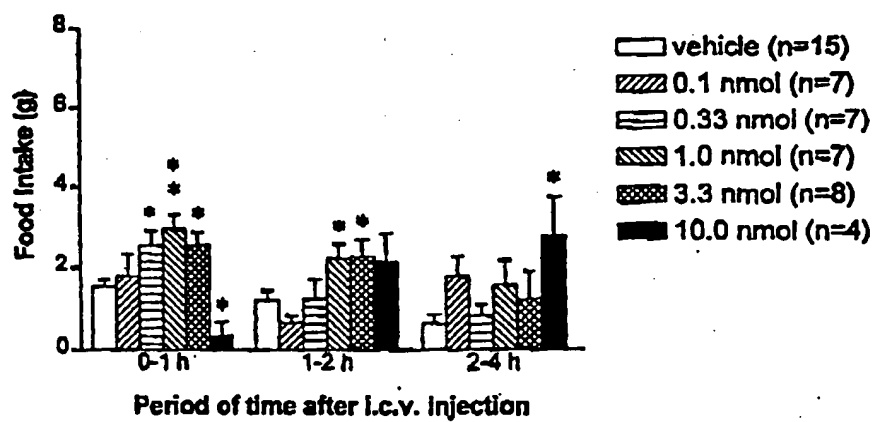
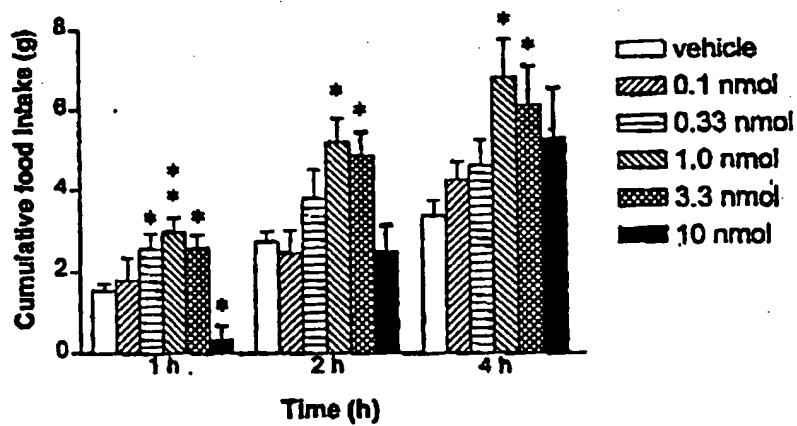
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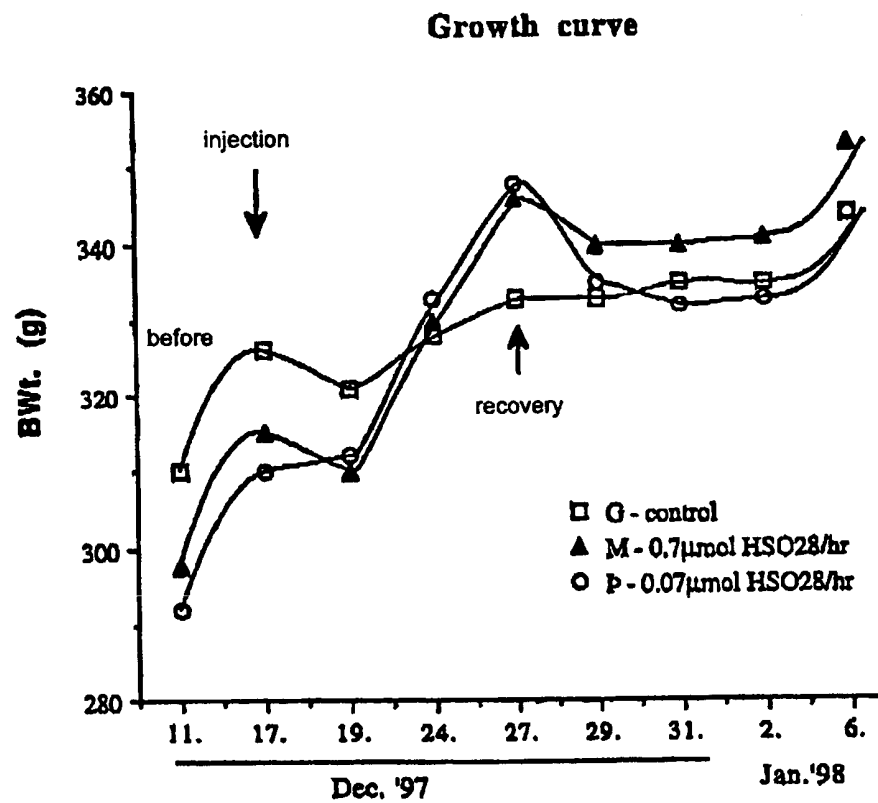
10. A compound according to any of claims 1-7, wherein a label is incorporated or to which a label is attached.

11. The compound of claim 10, wherein the label includes radioactive atom or is biotin.

35

12. Use of a compound according to any of claims 1-7 and 10-11 for radioligand binding, quantification of melanocortin receptors, localisation of melanocortin receptors in tissues, receptor autoradiography, photoaffinity labeling, and/or imaging based on any of gamma photon detection method, scintigraphy, PET and SPECT.
13. Use of a compound according to any of claims 1-7 for the treatment of a condition mediated by an MC-receptor.
14. Use according to claim 13, wherein the MC-receptor is comprised by the group consisting of MC1, MC2, MC3, MC4, MC5.
15. Use according to claim 13 or 14, wherein the condition is selected from conditions related to eating, body weight, anorexia, anorexia nervosa, bulimia, anorexia in elderly, cachexia, cancer chemotherapy, anorexia in patients having cancer, cachexia in patients having cancer, motivation, learning, memory, behaviour, inflammation, body temperature, pain perception, blood pressure, heart rate, vascular tone, natriuresis, brain blood flow, nerve growth, placental development, aldosterone synthesis and release, thyroxin release, spermatogenesis, ovarian weight, prolactin and FSH secretion, uterine bleeding in women, sebum and pheromone secretion, blood glucose levels, intrauterine foetal growth, as well as other events related to parturition, and neural damage.

Fig. 1a**Fig. 1b**

**Fig. 2**

INTERNATIONAL SEARCH REPORT

International application No.

PCT/SE 98/00270

A. CLASSIFICATION OF SUBJECT MATTER

IPC6: C07K 14/68, C07K 7/06, A61K 38/34

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC6: C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

REG, CAPLUS, WPI, MEDLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 4485039 A (VICTOR J. HRUBY ET AL), 27 November 1984 (27.11.84), see claims 6-8 --	1-7,9-15
A	US 4649191 A (VICTOR J. HRUBY), 10 March 1987 (10.03.87) --	1-7,9-15
A	J.Med.Chem., Volume 38, 1995, Carrie Haskell-Luevano et al, "Design, Synthesis, Biology, and Conformations of Bicyclic alpha-Melanotropin Analogues" page 1736 - page 1750 -- -----	1-7,9-15

☐ Further documents are listed in the continuation of Box C.☒ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

28 May 1998

Date of mailing of the international search report

08-06-1998

Name and mailing address of the ISA/

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/SE 98/00270

Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: 8
because they relate to subject matter not required to be searched by this Authority, namely:
See PCT Rule 39.1(iv): Methods for treatment of the human or animal body by surgery or therapy, as well as diagnostic methods.
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT
Information on patent family members

29/04/98

International application No.

PCT/SE 98/00270

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 4485039 A	27/11/84	NONE	
US 4649191 A	10/03/87	NONE	

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